Quantification of the anti-neoplastic polyacetylene falcarinol from carrots in human serum by LC-MS/MS

Jakobsen, Ulla; Kobæk-Larsen, Morten; Kjøller, Karoline D.; Antonsen, Steen; Baatrup, Gunnar; Trelle, Morten B.

Published in:
Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences

DOI:
10.1016/j.jchromb.2022.123440

Publication date:
2022

Document version:
Final published version

Journal of Chromatography B 1210 (2022) 123440

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Quantification of the anti-neoplastic polyacetylene falcarinol from carrots in human serum by LC-MS/MS

Ulla Jakobsen [a], Morten Kobæk-Larsen [b,c], Karoline D. Kjøller [b,c], Steen Antonsen [a], Gunnar Baatrup [b,c], Morten B. Trelle [a,b,*]

[a] Department of Biochemistry, Odense University Hospital, Svendborg Hospital, Svendborg, Denmark
[b] Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[c] Department of Surgery, Odense University Hospital, Odense, Denmark

ARTICLE INFO

Keywords:
Falcarinol
Antineoplastic
Polyacetylenes
Gastrointestinal cancer
Mass spectrometry
LC/MS-MS

ABSTRACT

Falcarinol is a polyacetylene which is found in carrots and known to have anti-neoplastic properties in rodents. Research in the bioactivity of falcarinol in humans require methods for quantification of falcarinol in human serum. Here we report the development of an LC-MS/MS method and its use to measure serum falcarinol concentrations in humans following intake of a carrot product. Falcarinol was measured by LC-MS/MS using the *m/z* 268 to *m/z* 182 mass transition. Six calibrator levels (0.2–20 ng/mL) and 3 control levels (0.4, 2 and 8 ng/mL) were prepared by addition of falcarinol to human serum pools. Linearity of the developed method was good with a mean R^2 of 0.9942. Within-day, between-day and total coefficients of variation were 6.9–13.1%, 4.1–5.0% and 8.1–14.0%, respectively. The limits of detection and quantitation were 0.1 and 0.2 ng/mL, respectively, matrix effects 84.2%, recovery 101.4–105.4% and carry-over −0.24–0.07%. Serum falcarinol concentrations were measured in 18 healthy volunteers prior to and at 9 time-points following intake of a carrot product. Falcarinol concentrations peaked at the 1-hour time-point after intake in 15 out of 18 volunteers and declined according to a single exponential decay function with an aggregate $t_{½}$ of 1.5 h.

In conclusion, an LC-MS/MS method for quantification of falcarinol in human serum with acceptable performance was developed and used to measure falcarinol concentrations following intake of a carrot product.

1. Introduction

Falcarinol (FaOH) (Fig. 1A) is primarily found in carrots and other plants of the Apiaceae family, where the compound can act as a phytoalexin [1]. It is also known to have biological activities in cells and mammals. It has been shown to have e.g. a hormesis effect [2–4], cytotoxicity against cancer cell lines *in vitro* [2,5–8] and an anti-neoplastic effect in a rat model for colorectal cancer [9–11]. Freeze dried carrots and purified FaOH reduce the growth rate of neoplasms in the colon of rats primed to develop colon cancer with the carcinogenic compound azoxymethane [9]. Furthermore, both the number of aberrant crypt foci and adenomas in the colon were reduced in rats following treatment with FaOH and the related falcarindiol [10].

The established cancer-preventive effect of FaOH in animal models has ignited a profound interest in investigation of putative cancer preventive effects of FaOH in humans. However, little is known about the uptake and metabolism of FaOH from the dietary source in the human body. Methods for quantitation of FaOH in human plasma following intake of carrots or carrot product are scarce and lacking sufficient detail to be reproduced [12,13]. The same is also the case for a method for quantification of FaOH in murine plasma, where the FaOH levels furthermore are higher than those found in human serum following oral administration [14]. Quantitative methods for determination of FaOH in plants or products thereof by a number of different methods, such as HPLC [2,5,9,15–19], gas chromatography (GC) [20,21], gas chromatography-mass spectrometry (GC–MS) [22] and mass spectrometry (MS) [10,23,24] have been described in the literature. However, FaOH-levels are orders of magnitude higher in plant extracts compared to human serum, wherefore these methods generally are not sensitive enough for our purpose. Sensitivity in the low ng/mL

Abbreviations: EtOH, Ethanol; FaOH, falcarinol; GC, Gas Chromatography; HPLC, High Pressure Liquid Chromatography; MeCN, Acetonitrile; MeOH, Methanol; MS, Mass Spectrometry; SIM, Single ion monitoring; SRM, Single Reaction Monitoring.

* Corresponding author at: Department of Biochemistry, Odense University Hospital, Svendborg Hospital, Baagøes Allé 15, 5700 Svendborg, Denmark.
E-mail address: morten.beck.trelle@rsyd.dk (M.B. Trelle).

https://doi.org/10.1016/j.jchromb.2022.123440
Received 2 May 2022; Received in revised form 23 August 2022; Accepted 27 August 2022
Available online 6 September 2022

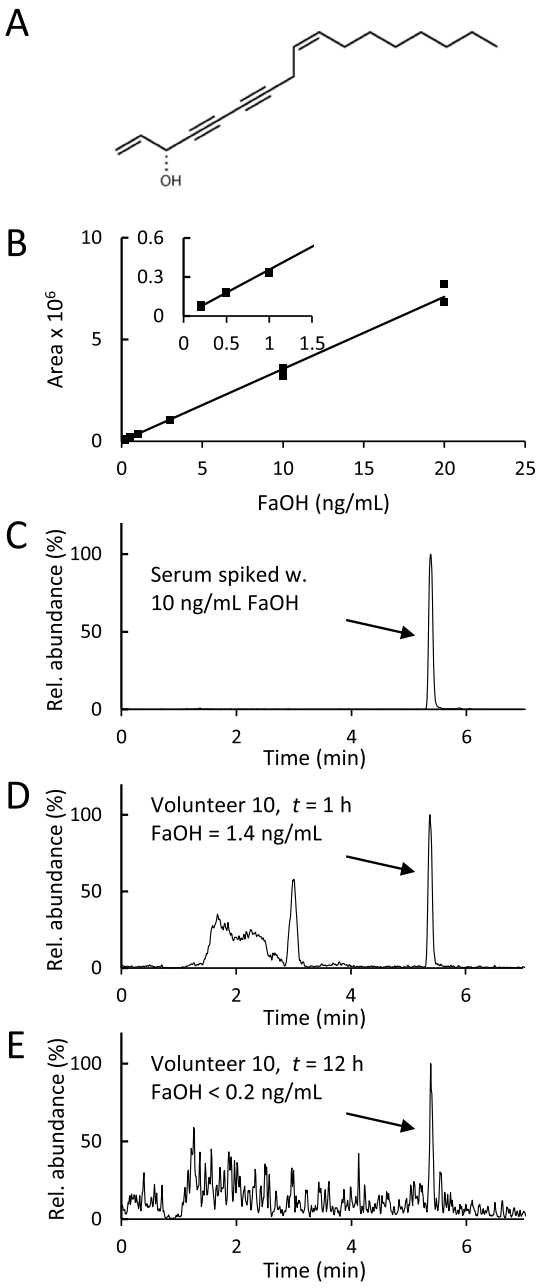

Fig. 1. A) Structure of FaOH. B) Representative calibration curve with zoom of the low range. LC-MS/MS chromatograms of samples with 10 ng/mL FaOH spiked into a serum pool (C) and samples from volunteer 10 obtained 1 (D) and 12 h(s) (E) after carrot product intake. The FaOH peak area in (E) would correspond to a concentration below the LOQ of 0.2 ng/mL.

range is needed and high pg/mL range desired.

We have therefore developed a method for the analysis of FaOH in human serum and used it to investigate the uptake and clearance from human serum after oral intake of a carrot product.

2. Materials and Methods

2.1. Chemicals

Falcarinol (3*R*,9Z-heptadeca-1,9-diene-4,6-diyn-3-ol) was purchased as a solution of 10 mg/mL (quantitative accuracy of −2% to + 5% and purity of 85.9%) in ethanol (EtOH) from Cayman Chemical Company and diluted to a working stock solution of 10 μg/mL FaOH in EtOH, factoring in the purity. LC-MS-grade acetonitrile (MeCN) and methanol (MeOH) was purchased from Honeywell, HPLC-grade abs. EtOH from Th.Geyer, and HPLC-grade acetone, isopropanol and formic acid (purity 98–100%) from Merck. Laboratory grade water was produced in-house in a custom build purifications system from BWT. Serum used for preparation of calibrators, controls and for method validation in general was left-over, anonymized patient material from the Department of Biochemistry at Odense University Hospital, Svendborg Hospital.

2.2. Calibrators and Controls

Serum rather than pure solvent was chosen as the matrix for calibrators and controls due to a substantial matrix effect in serum (see section 2.8 and 3.3). As calibrator and control materials for analysis of FaOH in human serum are not commercially available, these were prepared in-house by spiking of human serum pools. Samples used were centrifuged at 2404 g for 4 min. at 20 °C prior to pooling. For each pool, a stock solution in serum was made by adding 25 μL of a solution of 10 μg/mL FaOH in EtOH to 475 μL serum, giving a FaOH concentration of 500 ng/mL. Calibrators and controls were then prepared by mixing appropriate amounts of these stock solutions with serum from the same pool to achieve calibrators with final FaOH concentrations of 1, 3, 10 and 20 ng/mL and controls with final FaOH concentrations of 2 and 8 ng/mL. Calibrators with the concentrations of 0.2 and 0.5 ng/mL were made by dilution of the 20 ng/mL calibrator with serum, and the control with 0.4 ng/mL were made by dilution of a sample of 40 ng/mL. Three sets of calibrators were prepared from two different serum pools and four different control sets were prepared from three different serum pools. All serum pools were analyzed un-spiked to assess the background FaOH level and only serum pools with no visible sign of a chromatographic FaOH peak, corresponding to a falcarinol level well below the determined LOD of 0.1 ng/mL, were used. Handling of falcarinol solutions at room temperature in the laboratory was kept to a minimum and amber glass vials and aluminum foil was used to keep the materials light-protected. Calibrators and controls were stored at −20 °C in the dark.

2.3. Sample preparation

The same sample preparation protocol was utilized for all sample types (calibrators, controls, spiked serum, serum pools and unknown samples). Samples were centrifuged at 2404 g for 4 min. at 20 °C. Serum proteins were precipitated by mixing 200 μL of sample with 600 μL of MeCN in 1.5 mL Eppendorf tubes. The precipitated proteins were pelleted by centrifugation at 9920 g for 5 min. at 20 °C and the supernatant transferred to 1.5 mL amber glass vials prior to injection into the LC-MS/MS system. All calibrators and controls were prepared on the same day as the samples analyzed in the same bracket.

2.4. LC-MS/MS conditions

Samples were analyzed on a modified Transcend TLX1 HPLC-system coupled to a TSQ Quantum Ultra mass spectrometer from Thermo-Fisher Scientific. 80 μL of sample was injected into a 100 μL loop and transferred to a Kinetex® 2.6 μ C18 100 Å, 150 × 3.00 mm column (Phenomenex), maintained at a temperature of 30 °C. Solvent A was water containing 0.1% formic acid and solvent B was MeCN containing 0.1% formic acid. Washing of the column was done with solvent C composed of 40% MeOH, 40% isopropanol, 10% acetone and 10% water. Gradient elution of FaOH and washing/reconditioning of the column was done with the following steps: from 60 to 95% B in 4 min and 23 s, at 95% B for 1 min and 30 s, at 100% C for 1 min and at starting conditions for 2 min and 30 s. The solvent flow was 0.5 mL/min. The mass spectrometer was operated in the positive ion mode with the following ion source settings: spray voltage of 3500 V, vaporizer temperature of 350 °C, sheath gas pressure of 20 psi, ion sweep gas pressure of 0 (arbitrary

units), auxillary gas pressure of 25 (arbitrary units) and capillary temperature of 300 °C. FaOH was detected using single reaction monitoring (SRM) of the *m/z* 268 to *m/z* 182 transition, with argon as collision gas and a collision energy of 25 eV.

2.5. Analysis batch design and calibration

To counteract intra-serial drift in mass spectrometric ionization response a "bracketing" of samples with calibrators was used. Each analysis batch was divided into 2–3 brackets of 20 samples surrounded by calibrators and controls, such that each bracket consisted of calibrators, controls, 20 samples, controls and calibrators. The calibrators ending one bracket was the first calibrators in the following bracket (i.e. the calibrators were only analyzed once between 2 × 20 samples).

Calibration curves relating chromatographic peak areas to target values of calibrators were generated using linear regression with 1/x-weighing of residuals and not forced through the origin. Only calibration brackets with $R^2 > 0.99$ (rounded) were accepted.

2.6. Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Linearity was assessed on the basis of calibration data, e.g. means of slope, intercept, R^2-values and residuals ± standard deviations. The limit of detection (LOD) and the limit of quantification (LOQ) was assessed by 10 individually prepared replicate analyses of a serum pool spiked to a nominal FaOH level of 0.1 and 0.2 ng/mL, respectively, on four different days. The LOD was calculated as 5x the standard deviation for each day and as a mean between the four days. The LOQ was defined as the lowest value with a mean imprecision less than 25% and mean deviation from target of less than 25%.

2.7. Precision

Intra- and inter-day precision was determined from 4 to 6 replicate analysis of control samples on 13 different days. Within-day CV's were calculated as a weighted mean of the CV obtained within each analysis day using the number of measurements each day as weights. Between-day CV's were calculated from a weighted SD of within-day means using the number of measurements each day as weights. Total CV was calculated from the SD and mean using all measurements from all days.

2.8. Matrix effects and Recovery

The matrix effect was evaluated as the percentage-fraction of FaOH measured (peak areas) when spiked into serum samples, after sample preparation, relative to FaOH in pure solvent. Ten different serum samples were centrifuged at 2404 g for 4 min. From each samples, aliquots of 2 × 200 μL were removed and precipitated with MeCN as described above. To 600 μL of the supernatant was added either 7.5 μL of a solution of 105 ng/mL FaOH in EtOH (giving a theoretical concentration of 5 ng/mL FaOH) or 7.5 μL of abs. EtOH before analysis. A comparable sample of FaOH in pure solvents were prepared by mixing 200 μL H_2O, 600 μL MeCN and 10 μL of the 105 ng/mL FaOH solution. This study was repeated on 3 separate days using new serum samples. The matrix effect was calculated as the mean of the matrix effect (%) obtained on all analyzed sera according to the formula below:

$$Matrix\ \ effect(\%) = \frac{Area_{spiked} - Area_{unspiked}}{Area_{solvent}} \times 100\%$$

Furthermore, the mean SD of the matrix effect (%) of the 10 different serum samples analyzed each day was calculated to evaluate the variation of matrix effect among different sera.

Recovery was evaluated as the percentage-fraction of FaOH measured (ng/mL) when spiked into serum samples prior to sample preparation, relative to the theoretically added amount of FaOH. As control samples were prepared by spiking serum pools with FaOH, these were used to calculate recovery. Recovery was calculated as the mean ± SD of the recovery (%) obtained on each of the 13 days of control sample analysis according to the following formula:

$$Recovery(\%) = \frac{Conc._{spiked} - Conc._{unspiked}}{Conc_{theoretically\ \ added}} \times 100\%$$

2.9. Carry-over

A serum sample spiked to a FaOH concentration of 20 or 40 ng/mL was analyzed in triplicate (H1, H2 and H3) followed by the analysis of a sample with a FaOH concentration of 0.4 ng/mL in triplicate (L1, L2 and L3). The carry-over was calculated as: $Carry - over\ \ (\%) = \frac{L1-L3}{H3-L3} \times 100\%$

This study was repeated 2 times for the 40 ng/mL samples and 5 times for the 20 ng/mL sample. The results are reported as mean carry-over% ± SD.

2.10. Bioavailability

Uptake and clearance of FaOH in human serum following intake of a carrot product were studied in 18 healthy volunteers. The study was conducted within the guidelines of the Helsinki Declaration and was approved by the local committee on health research ethics (Projekt-ID: S-20200084).

Ten women and eight men aged 21–68 years, were included. The volunteers were asked to exclude carrots and other vegetables known to contain FaOH from their diet 24 h before the test and otherwise follow their normal diet without fasting. The study was conducted at Odense University Hospital, Svendborg Hospital. Upon participation each volunteers gave their informed written consent.

Blood samples were drawn from each volunteer in the morning shortly before carrot intake and thereafter 1, 2, 2.5, 3, 4, 6, 8, 10 and 12 h after intake. Due to the repeated samplings, most volunteers preferred to have the samples drawn through a peripheral intravenous cannula from which 4 mL of blood was discarded, before samples were collected in a 6 mL BD Vacutainer CAT (Clot Activator Tube) tube (Becton Dickinson). The remaining volunteers had blood samples drawn by repeated venipuncture. Samples were left for coagulation for at least 30 min., centrifuged at 2000 × g for 10 min. at 20 °C and serum frozen at −80 °C until analysis.

The carrots used were grown organically at Danroots A/S, Bjerringbro, Denmark. Tops and bottoms were removed from fresh, washed carrots, which were then shredded, freeze-dried and prepared into powder (European Freeze-Dry, Kirke Hyllinge, Denmark). The powder was packed in sealed aluminum foil pouches and stored at – 30 °C until use. According to literature, lyophilization does not affect FaOH content in carrots [24]. Freeze-dried carrot powder slurries were prepared on the day of use and contained 30 g of freeze-dried carrot powder (cv. Night Bird F1 hybrid) and 500 mL of tap water. The amount of 30 g of freeze-dried carrot powder correspond to approximately 300 g raw carrot (2–3 average sized carrots).

Serum concentrations of FaOH were plotted as a function of time for each volunteer. Assuming a one compartment volume of distribution (V_d), single exponential decay functions were fitted to the declining phase of these serum FaOH concentration measurements:

$$c(FaOH) = N \times e^{-kt}$$

where *N* represent the intercept with the y-axis, *k* the rate constant and *t* the time (h).

From the rate constant *k* the half-life of exponential decay ($t_{½}$) can be calculated as:

$$t_{½} = \frac{ln(2)}{k}$$

The measured FaOH concentrations for each volunteer were

normalized to the peak value and average normalized concentrations among all 18 volunteers were plotted as a function of time (Fig. 2). An exponential decay function, of the form above, was fitted to the declining part of this curve to generate an aggregate rate constant and $t_{½}$ using data from all volunteers.

3. Results:

3.1. LC-MS/MS detection of FaOH

A range of LC-MS/MS method parameters were explored in the initial phase of the method development (see discussion), including which mass transitions to choose for quantitation of falcarinol. The most abundant ion signal observed correspond to protonated falcarinol with an MeCN adduct and a loss of water ($[M - H_2O + MeCN + H]^+$ at *m/z* 268, Supplementary Fig. 1) and was chosen as precursor ion in the SRM method. The fragment ion at *m/z* 182 was most intense and chosen as product ion.

The developed LC-MS/MS method enables detection of FaOH as a temporarily increasing and decreasing chromatographic peak, base-line separated from apparent interferences, when samples containing FaOH in pure solvents or spiked into human serum are analyzed (Fig. 1C). Furthermore, a similar peak is produced at the exact same retention time, when human serum samples are analyzed following ingestion of 500 mL of carrot powder slurry known to contain FaOH (Fig. 1D and 1E).

3.2. Calibration, linearity, LOD and LOQ

For 28 calibration brackets, performed on 13 different days, good linearity was observed in the investigated concentration range with the following parameters (Table 1): The mean R^2 was 0.9942 ± 0.0029 and the mean residuals from target were less than 7% at all levels and without concentration-dependent bias. The mean slope was 2.67 × 10^5 ± 0.61 × 10^5 Area × mL × ng^{-1}. The mean intercept with the x-axis was −0.032 ± 0.036 ng/mL. The observed variation in calibration parameters reflect the variation in mass spectrometric ionization response. A representative calibration curve is shown in Fig. 1B and mean residuals in Table 2. The LOD was 0.1 ng/mL and the LOQ was 0.2 ng/mL.

3.3. Imprecision, matrix effects, recovery and carry-over

Within-day CV́s (6.9–13.1%) were considerably higher compared to between-day CV's (4.1–5.0%), at all control levels, and thus constituted the main contributor to the total CV's (8.1–14.0%) (Table 3).

Matrix effects was assessed in 10 different sera on 3 different days and found to be 84.2 % (16.8% negative interference). An average SD of 7.9% among the 10 sera tested each day was observed. Considering within-day CV́s of similar magnitude there is no indication from these data that the matrix effect differs significantly from individual to individual. Recovery was assessed from control samples and found to be 104.5% ± 6.3% at 0.4 ng/mL, 101.4% ± 5.5% at 2.0 ng/mL and 105.4% ± 7.0% at 8.0 ng/mL. Carry-over was −0.24% ± 0.77% when 40 ng/mL injection were followed by 0.4 ng/mL injections and 0.07% ± 0.45% when 20 ng/mL injections were followed by 0.4 ng/mL injections.

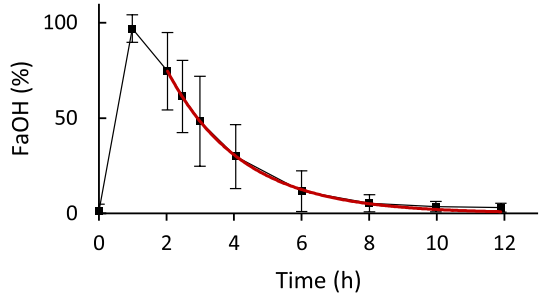

Fig. 2. Relative serum FaOH levels following intake of a carrot product. FaOH concentrations were normalized to the maximum value for each of the 18 participants. The black squares, connected by a thin black line, represent the average relative FaOH concentration for all 18 participants. Whiskers indicate standard deviations. A single declining exponential function is fitted to the data (red line).

Table 1
Calibration parameters. Linear regression.

Calibration Parameter	Value
Number of calibration brackets	28
Mean slope ± SD (10^5 Area × mL × ng^{-1})	2.67 ± 0.61
Mean y-axis intercept ± SD (10^5 Area)	0.076 ± 0.087
Mean x-axis intercept ± SD (ng × mL^{-1})	−0.032 ± 0.036
Mean R^2 ± SD	0.9942 ± 0.0029

Calibration curves relating chromatographic peak areas to target values of calibrators were generated using linear regression with 1/x-weighing of residuals. Mean slope, mean intercept with y- and x-axis and mean R^2-values ± standard deviation of all parameters are indicated.

Table 2
Calibration parameters. Residuals.

Calibrator Levels	Target (ng × mL^{-1})	Mean residuals ± SD (ng × mL^{-1})
1	0.2	−0.01 ± 0.03
2	0.5	−0.03 ± 0.07
3	1	0.05 ± 0.16
4	3	0.21 ± 0.25
5	10	−0.07 ± 0.61
6	20	−0.15 ± 1.10

Mean residuals ± standard deviation for all calibrator levels.

Table 3
Imprecision.

Level (ng × mL^{-1})	Within-day CV%	Between-day CV%	Total CV%
0.4	13.1	5.0	14.0
2.0	6.9	4.3	8.1
8.0	8.7	4.1	9.6

Imprecision data obtained by repeated measurements of control samples. Within-day, between-day and total CV's are indicated.

3.4. Bioavailability

Serum FaOH levels were measured at 9 time-points following carrot product intake by 18 healthy volunteers. Peak FaOH level was measured at the 1-hour time-point following intake in 15 out of 18 volunteers and the peak concentration was 0.9–4.0 ng/mL (Table 4). A single exponential decay function was fitted to the declining phase of the serum FaOH concentration measurements for each individual and half-lifes ($t_{½}$) of the declining FaOH concentration were in the 0.8–2.1 h range (Table 4).

To estimate an aggregate t½ using data from all volunteers, average normalized FaOH concentrations were plotted as a function of time and fitted to a declining exponential decay function (Fig. 2), yielding a $t_{½}$ of 1.5 h.

Table 4
Falcarinol serum values following carrot product intake.

Volunteer	Peak falcarinol (ng/mL)	Peak time (h)	$t_{½}$ (h)
1	2.8	1	0.9
2	2.2	1	1.5
3	1.6	1	2.1
4	0.9	1	0.8
5	3.1	2.5	1.0
6	1.5	2	0.9
7	2.2	1	1.6
8	1.4	1.5	2.0
9	2.2	1	0.9
10	1.4	1	1.8
11	1.7	1	1.6
12	2.6	1	1.1
13	4.0	1	1.6
14	1.4	2	1.6
15	3.8	1	0.9
16	3.6	1	1.0
17	1.5	1	1.7
18	1.9	1	1.2

Peak falcarinol concentration, peak time and estimated half-life ($t_{½}$) of falcarinol clearance from serum in each of the 18 healthy volunteers.

4. Discussion

Falcarinol is known to have biological activity in cells and mammals, e.g. to reduce cell proliferation and neoplastic lesions in a rat model for colorectal cancer [9–11]. To study the bioactivity of FaOH in humans, it is advantageous to be able to determine the concentration of the compound in blood.

The analysis of FaOH in human plasma using LC-MS/MS has been described in the literature [12,13], but without sufficient details to be reproduced. For this reason, an LC-MS/MS method for measuring FaOH in human serum has been developed, characterized and used.

To find and optimize the conditions used for LC-MS/MS analysis, solutions of FaOH in solvent were used. Electrospray ionization in both positive and negative mode was investigated. The characterization of FaOH in the negative mode has been described in the literature [25]. However, as also described by others [24] this was not successful in our hands, wherefore we continued with the positive mode. Acetonitrile and methanol, both containing 0.1 % formic acid, were tested as mobile phases. In both, solvent adducts as well as dimeric and higher adducts were seen, as described previously [24]. Due to fewer and less intense polymer signals observed, and better sensitivity at low FaOH concentrations, we decided to use MeCN as eluent instead of MeOH. When using MeCN, the adduct $[M - H_2O + MeCN + H]^+$ (m/z 268) was the most abundant ion and was chosen for analysis. This ion has also been observed and/or used for quantification of FaOH by others [19,23,24] by single ion monitoring (SIM) approaches. However, SIM was not sensitive enough for our human samples in which FaOH concentrations are much lower than in plant extracts and murine serum. A number of different single reaction monitoring (SRM) transitions were thus tested, and the transition m/z 268 to m/z 182 gave useful sensitivity and signal-to-noise ratio. A number of chromatographic parameters were explored during the method development, such as gradient length, injection volume and column length. Initial experimentation with a shorter C18 column was abandoned due to a very low intensity interfering peak in the m/z 268 to m/z 182 mass transition, with a chromatographic peak partially overlapping with that of FaOH. By shifting to the 150 mm C18 column (see section 2.4) this interference was chromatographically separated by approximately 5 peak widths from the FaOH peak.

An isotope-labelled FaOH would be the internal standard of choice, but as this is not commercially available the method was developed without an internal standard. To compensate for drift in mass spectrometric ionization response during analysis, each series was divided into 2–3 “brackets” (the number dependent on the number of samples to be analyzed) each beginning and ending with analysis of the six calibrators and each consisting of 20 samples with controls analyzed before and after these.

The developed LC-MS/MS method was subjected to a method performance characterization. Due to the relatively low concentration of FaOH in human serum following oral intake of carrot products, the linearity was only assessed up to 20 ng/mL, as this is more than adequate for this purpose. To improve the determination of especially low concentrations of FaOH, more calibrators with low concentrations (0.2, 0.5, 1 ng/mL) were used. The achieved LOD and LOQ of 0.1 and 0.2 ng/mL, respectively, were acceptable as well as a total CV of less than 15% at all control levels. The matrix effect was 84.2 %, which is acceptable, especially considering the absence of an internal standard, and the difference in matrix effect between different sera was no greater than expected from analytical variation in itself. Only negligible carry-over values were found.

The method was used to study the uptake and clearance of FaOH in human serum after intake of a carrot product corresponding to 2–3 average sized carrots. A rapid increase in FaOH levels were seen, with peak values one hour after intake, suggesting a rapid transfer of FaOH from the digestive tract and into circulation. Clearance of FaOH from serum also occurred fast, with levels declining below detection levels after 8–10 h. The maximum values of FaOH obtained varied in the range 0.9–4.0 ng/mL (Tabel 4). As blood was sampled in 1 h intervals around the peak values, it is possible that the true serum peak values were higher and occurred either before or after the observed peak value. The fast uptake and clearance found here is consistent with literature, where a maximum concentration of FaOH was reached 2 h after intake of carrot juice and almost no FaOH could be detected after 8 h[13]. The observed range of peak FaOH levels may be caused by variation in absorption from the gut among individuals or variation in the volume of distribution (V_d) of FaOH. However, more frequent samples around the peak time might have given better estimates of the true peak value and reduced the observed variation between volunteers. The aggregate half-life of FaOH clearance estimated from fitting of an exponential decay function to the average normalized data-points from all volunteers was found to be 1.5 h (Fig. 2). The excellent agreement between data points and the fitted exponential decay function provides support for the assumption of a one-compartment V_d for FaOH.

The developed method enables important future studies on the bioavailablity and bioactivity of FaOH and is thus a valuable tool for investigations of the putative anti-neoplastic effect of FaOH in humans. Here FaOH uptake and clearance following a single oral intake is reported. Future characterization of serum FaOH concentrations after daily intake of carrot products for longer periods is equally justified, as well as investigations of dosis-response relationships, both within and between individuals. Furthermore, uptake and clearance of FaOH from other carrot product forms (raw carrots, shredded carrots, carrot containing foods etc.), or other FaOH containing products, deserve independent investigation. The method may also facilitate mechanistic studies of FaOH bioactivity. Finally, correlations between serum FaOH concentrations and colorectal cancer status measures may thus prove valuable when testing hypotheses of anti-neoplastic effects of FaOH in humans in future clinical trials.

In conclusion, an LC-MS/MS method for quantification of FaOH in serum is reported. The method enables a range of interesting future studies of the putative cancer preventive effects of FaOH.

Funding

The study was supported by the Research Fund of Odense University Hospital (122-A5125) and The Research Fund of Region of Southern Denmark (A515). Danroots A/S supplied carrots at no charge.

CRediT authorship contribution statement

Ulla Jakobsen: Conceptualization, Methodology, Investigation,

Validation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Morten Kobæk-Larsen:** Conceptualization, Investigation, Funding acquisition, Writing – review & editing. **Karoline D. Kjøller:** Investigation, Writing – review & editing. **Steen Antonsen:** Conceptualization, Supervision, Writing – review & editing. **Gunnar Baatrup:** Conceptualization, Supervision, Writing – review & editing. **Morten B. Trelle:** Conceptualization, Methodology, Validation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Technicians Anette Holz Gregersen, Kis Søderholm Christensen and Mette Brandt Andreasen assisted with blood sampling. The 18 volunteers participated without compensation. They are all thanked for their contribution.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2022.123440.

References

[1] C. Dawid, F. Dunemann, W. Schwab, T. Nothnagel, T. Hofmann, Bioactive C_{17}-Polyacetylenes in Carrots (Daucus carota L.): Current Knowledge and Future Perspectives, J. Agric. Food Chem. 63 (42) (2015) 9211–9222.
[2] S.L. Hansen, S. Purup, L.P. Christensen, Bioactivity of falcarinol and the influenceof processing and storage on its content in carrots (Daucus carota L), J. Sci. Food Agric. 83 (10) (2003) 1010–1017.
[3] S. Purup, E. Larsen, L.P. Christensen, Differential Effects of Falcarinol and Related Aliphatic C17-Polyacetylenes on Intestinal Cell Proliferation, J. Agric. Food. Chem. 57 (18) (2009) 8290–8296.
[4] J.F. Young, S.J. Duthie, L. Milne, L.P. Christensen, G.G. Duthie, C.S. Bestwick, Biphasic effect of falcarinol on caco-2 cell proliferation DNA damage, and apoptosis, J. Agric. Food Chem. 55 (3) (2007) 618–623.
[5] C. Zidorn, K. Jöhrer, M. Ganzera, B. Schubert, E.M. Sigmund, J. Mader, R. Greil, E. P. Ellmerer, H. Stuppner, Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities, J. Agric. Food Chem. 53 (7) (2005) 2518–2523.
[6] H. Matsunaga, M. Katano, H. Yamamoto, H. Fujito, M. Mori, K. Takata, Cytotoxic activity of polyacetylene compounds in Panax ginseng C. A. Meyer, Chem. Pharm. Bull. (Tokyo) 38 (12) (1990) 3480–3482.
[7] Y. Um, C.-S. Kong, J. Lee, Y.A. Kim, T.-J. Nam, Y. Seo, Evaluation of chemical constituents from Glehnia littoralis for antiproliferative activity against HT-29 human colon cancer cells, Process Biochem. 45 (2010) 114–119.
[8] M.W. Bernart, J.H. Cardellina 2nd, M.S. Balaschak, M.R. Alexander, R. H. Shoemaker, M.R. Boyd, Cytotoxic falcarinol oxylipins from Dendropanax arboreus, J. Nat. Prod. 59 (8) (1996) 748–753.
[9] M. Kobaek-Larsen, L.P. Christensen, W. Vach, J. Ritskes-Hoitinga, K. Brandt, Inhibitory effects of feeding with carrots or (-)-falcarinol on development of azoxymethane-induced preneoplastic lesions in the rat colon, J Agric Food Chem 53 (5) (2005) 1823–1827.
[10] M. Kobaek-Larsen, R.B. El-Houri, L.P. Christensen, I. Al-Najami, X. Fretté, G. Baatrup, Dietary polyacetylenes, falcarinol and falcarindiol, isolated from carrots prevents the formation of neoplastic lesions in the colon of azoxymethane-induced rats, Food Funct. 8 (3) (2017) 964–974.
[11] M. Kobaek-Larsen, G. Baatrup, M. KhataeiNotabi, R.B. El-Houri, E. Pipó-Ollé, E. Christensen Arnspang, L.P. Christensen, Dietary Polyacetylenic Oxylipins Falcarinol and Falcarindiol Prevent Inflammation and Colorectal Neoplastic Transformation: A Mechanistic and Dose-Response Study in A Rat Model, Nutrients 11 (9) (2019).
[12] L.P. Christensen, K. Brandt, Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis, J. Pharm. Biomed. Anal. 41 (3) (2006) 683–693.
[13] K. Brandt, L.P. Christensen, J. Hansen-Møller, S.L. Hansen, J. Haraldsdottir, L. Jespersen, S. Purup, A. Kharazmi, V. Barkholt, H. Frøkiær, M. Kobaek-Larsen, Health promoting compounds in vegetables and fruits: A systematic approach for identifying plant components with impact on human health, Trends Food Sci. Technol. 15 (2004) 384–393.
[14] H. Tashkandi, A. Chaparala, S. Peng, M. Nagarkatti, P. Nagarkatti, A. A. Chumanevich, L.J. Hofseth, Pharmacokinetics of Panaxynol in Mice, J. Cancer Sci. Clin. Ther. 4 (2) (2020) 133–143.
[15] L.P. Christensen, S. Kreutzmann, Determination of polyacetylenes in carrot roots (Daucus carota L.) by high-performance liquid chromatography coupled with diode array detection, J. Sep. Sci. 30 (4) (2007) 483–490.
[16] U. Kidmose, S.L. Hansen, L.P. Christensen, M. Edelenbos, E. Larsen, R. Nørbæk, Effects of Genotype, Root Size, Storage, and Processing on Bioactive Compounds in Organically Grown Carrots (Daucus carota L.), J. Food Sci. 69 (9) (2004) S388–S394.
[17] C. Zidorn, S. Sturm, J.W. Dawson, J.W. van Klink, H. Stuppner, N.B. Perry, Chemosystematic investigations of irregular diterpenes in Anisotome and related New Zealand Apiaceae, Phytochemistry 59 (3) (2002) 293–304.
[18] J. Avalos, G.P. Fontan, E. Rodriguez, Simultaneous HPLC Quantification of Two Dermatotoxins, 5-Methoxypsoralen and Falcarinol, Healthy Celery, J. Liq. Chromatogr. 18 (10) (1995) 2069–2076.
[19] M. Søltoft, M.R. Eriksen, A.W.B. Träger, J. Nielsen, K.H. Laursen, S. Husted, U. Halekoh, P. Knuthsen, Comparison of Polyacetylene Content in Organically and Conventionally Grown Carrots Using a Fast Ultrasonic Liquid Extraction Method, J. Agric. Food. Chem. 58 (13) (2010) 7673–7679.
[20] A. Czepa, T. Hofmann, Quantitative studies and sensory analyses on the influence of cultivar, spatial tissue distribution, and industrial processing on the bitter off-taste of carrots (Daucus carota l.) and carrot products, J. Agric. Food Chem. 52 (14) (2004) 4508–4514.
[21] S. Nitz, M.H. Spraul, F. Drawert, C_{17} Polyacetylenic Alcohols as the Major Constituents in Roots of *Petroselinum crispum* Mill. ssp. *tuberosum*, J. Agric. Food Chem. 38 (1990) 1445–1447.
[22] P.A.G. Santos, A.C. Figueiredo, M.M. Oleveira, J.G. Barroso, S.G. Deans, J.J. C. Scheffer, Growth and essential oil composition of hairy root cultures of Levisticum officinale W.D.J. Koch (lovage), Plant Sci. 168 (2005) 1089–1096.
[23] E. Paulsen, T.H. Petersen, X.C. Fretté, K.E. Andersen, L.P. Christensen, Systemic allergic dermatitis caused by Apiaceae root vegetables, Contact Dermatitis 70 (2) (2014) 98–103.
[24] E.-M. Pferschy-Wenzig, V. Getzinger, O. Kunert, K. Woelkart, J. Zahrl, R. Bauer, Determination of falcarinol in carrot (Daucus carota L.) genotypes using liquid chromatography/mass spectrometry, Food Chem. 114 (3) (2009) 1083–1090.
[25] D.K. Rai, N.P. Brunton, A. Koidis, A. Rawson, P. McLoughlin, W.J. Griffiths, Characterisation of polyacetylenes isolated from carrot (Daucus carota) extracts by negative ion tandem mass spectrometry, Rapid Commun. Mass Spectrom. 25 (15) (2011) 2231–2239.