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Determination of fucoidan in rat plasma by HPLC and its application in pharmacokinetics

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Abstract: This is a new expanded method of determining the characterisation of fucoidan from *Laminaria japonica* (kelp) in rat plasma by high-performance liquid chromatography (HPLC) with fluorescence detection. We tagged fucoidan by fluoresce in isothiocyanate（FITC）for tracking and treated the plasma samples via protein precipitation with 10% trichloroacetic acid and methanol. Column chromatography separation was on a TSK-G4000sw column (7.8 mm × 300 mm, 5 μm) by elution with 0.15 M NaCl. The quantification of fucoidan was performed by HPLC with fluorescence detection. The results suggested that the calibration curve for fucoidan concentration was linear dependent in the limits of 0.5–100μg/mL. The lower limit of quantitation (LLOQ) was 0.5μg/mL and the lower limit of detection (LLOD) was 0.15μg/mL.The intra-day and inter-day precision values were less than 13%and the accuracy ranged from96.83 to 100.03% at 3 different concentrations. The fucoidan stability of rat plasma at different temperatures and time-points was estimated. The extraction efficiencies ranged from 93.33 to 96.53%and the matrix effect ranged from 92.67 to 95.83%. Method selectivity was evaluated as well. We successfully studied the pharmacokinetic of fucoidan in rat plasma after oral by the validated method. Fucoidan was administered to rats intravenously at a dose of 6 mg/kg and orally at a dose of 20 mg/kg. The C_{max} was 7.33μg/mL within 2 h by oral administration. The initial C_{max} was 75.59μg/mL. The bioavailability of fucoidan after oral administration to rats was 8.91%.

Keywords: Fucoidan, FITC labelling, *Laminaria japonica,* HPLC with fluorescence detection, validation.

INTRODUCTION

Fucoidans (fig. 1a) are a class of fucose-rich sulphated water-soluble polysaccharide extraction from brown seaweed (such as *Laminaria japonica*, *Costaria costata*, *Undaria pinnatitinda* and *Cladosiphon okamuranus*) (Lee *et al*., 2004; Pomin and Mourão 2008; Wang *et al*., 2010; Anastyuk *et al*., 2012) and echinoderms (such as sea cucumber and sea urchins) (Kariya *et al*., 2004; Cho *et al*., 2010). Fucoidans represent a family of sulphated hetero polysaccharides with different molecular weights, sulphate contents, fucose contents and monosaccharides, such as xylose, rhamnose, glucose, mannose, galactose, uronic acids, and arabinose (Pomin and Mourão 2008). It has been reported that fucoidans modulate the immune system, exhibit anti-carcinogenic properties, and possess many other anti-pathogenic, anti-inflammatory, anti-thrombotic, and anti-oxidant activities. They have been used for the treatment of liver and kidney disease (Cumashi*et al*., 2007; Khil'Chenko *et al*., 2011; Anastyuk *et al*., 2012; Chen *et al*., 2014; Janet *et al*., 2015).

Although many studies have reported the biological activity of fucoidans, few have examined its pharmacokinetics after ingestion. Generally speaking, the polysaccharides with high molecular weight are thought to have no uptake by oral. However, fucoidan from *Undaria pinnatifida* and *Cladosiphon okamuranus* can be absorbed by oral administration (Irhimeh *et al*., 2005; Tokita *et al*., 2014). Nagamine (2014) demonstrated the uptake and distribution of *Cladosiphon okamuranus* fucoidan (MW: 1927.2 kDa) in a rat model. The uptake was very low, and the transported fucoidan across Caco-2 cells was less than 0.01%, but it can be detected. An antibody to fucoidan was used for histological analysis which detected fucoidan in the small intestine, jejunal epithelium, mononuclear cells in the lamina propria and in sinusoidal non-parenchymal cells of the liver. The uptake of fucoidan by Kupffer cells was also found in the rats livers (Nagamine *et al*., 2014).

Laminaria japonica is common seafood that is widely cultured in China and many other countries. It has been documented as a therapeutic agent in traditional Chinese medicine. Fucoidan (MW: 100kDa) from *Laminaria japonica* is widely used in functional foods, health products, and dietary supplements, and has been shown to provide positive health benefits (Wang *et al*., 2010; Fitton *et al*., 2015). Thus, it is important to study the pharmacokinetics of fucoidan from food such as *Laminaria japonica*.

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Several analytical methods for quantitative tracking of fucoidan have been described, including immuno-cytochemistry (Irhimeh *et al*., 2005; Tokita *et al*., 2014), chromatography (Zhao *et al*., 2016), electrochemical methods (Kim *et al*., 2015) and dyes (Lee *et al*., 2012). However, many components in plasma can interfere with detection using these methods and sample treatment in plasma is very complicated. High-performance liquid chromatography (HPLC) with fluorescence detection can avoid this interference and simplify the treatment processing of the sample in plasma. It is necessary to establish a new method to detect the fucoidan in biological samples by HPLC with fluorescence.

In this paper, dextran (fig. 1b) was used as an internal standard (IS). A new method of quantitation detection of fucoidan in rat plasma by HPLC with fluorescence was established. The pharmacokinetics of fucoidan from *Laminaria japonica* in rats after oral administration was investigate during this new analytical method.

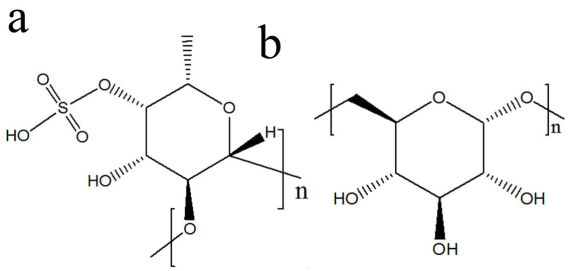


Fig. 1: Chemical structure of (a) fucoidan and (b) dextran

MATERIALS AND METHODS

Fucoidan from *Laminaria japonica* (molecular weight: 100 KDa) supplied by Weihai International Biotechnology Research and Development Centre at Shandong University (Weihai, China);
FITC (fluorescein isothiocyanate): Solarbio, Beijing, China;
NaBH3CN: Aladdin, Shanghai, China;
FITC-dextran (molecular weight: 70 KDa): Shanghai Ziqi Biological Technology Co., Ltd., Shanghai, China;
Trichloroacetic acid and methanol: Shanghai Shanpu Chemical Industry Co., Ltd., Shanghai, China;
EDTA and NaCl: RuijinTe Chemical Industry Co., Ltd., Tianjin, China.
Male Wistar rats, 250-300 g, from the Animal centre of Shandong University (Jinan, China).

Instrument
Fluorospectro photometer: LS45, Perkinelmer, USA;
Ultra-pure water: Milli-Q system, Millipore, MA, USA;
Water Purification Systems: Millipore-Q, Millipore, USA;
HPLC (high performance liquid chromatograph): Agilent 1100, Agilent, USA;
Chromatographic column: TSK-G4000sw, Agilent, USA;
PH meter: Orion 3-Star, Thermo, USA;
Pipette: Eppendorf, Germany;
Centrifuge: 5810R, Eppendorf, Germany

Preparation of fucoidan FITC
Synthesis of fucoidan tyramine
The fucoidan (400 mg) was dissolved in phosphate buffer (15mL, 0.2mol/L, pH = 8). Tyramine (400 mg) and sodium cyanoborohydride (150 mg) were added successively in a test tube. The mixture was incubated at 37^{o}C for 96h and shaken frequently. The solution was then centrifuged to obtain the supernatant. The supernatant was deposited by the addition of anhydrous ethanol to a final concentration of 80% (v/v) and placed at -4^{o}C for 12h. The precipitate was then collected by centrifugation (Eppendorf, Germany).

Synthesis of fucoidan – tyramine – FITC
The fucoidan –tyramine (200mg) was dissolved in $NaHCO_3$ (10 mL of 0.5 mol / L). The mixture was briefly vortexed to dissolve completely and then the FITC (25 mg) was added. The mixture was incubated in a shaker (speed 150) at 37^{o}C for 24 hours.

The reaction solution was centrifuged to obtain the supernatant. Anhydrous ethanol was then added to the supernatant to a final80% (v/v) concentration. The solution was kept at -4° C for 12h and collected the precipitate by centrifugation.

Standard and quality control samples
FITC-fucoidan stock solution: The FITC-fucoidan was dissolved in ultra-pure water to prepare2 mg/mL stock solutions and kept at 4^{o}C.

IS (FITC-dextran) stock solutions: The FITC-dextran was dissolved in ultra-pure water to prepare2 mg/mL stock solution and kept at 4^{o}C.

Standard plasma sample of FITC-fucoidan: FITC-fucoidan stock solution was diluted with blank plasma to 1 mg/mL.

Quality control (QC) plasma samples: The QC plasma samples of FITC-fucoidan were prepared at eight different concentrations 0.5, 5, 10, 20, 40, 60, 80 and 100μg/mL. There was 20μg/mL IS in every standard calibration sample. The QC samples were kept at -40^{o}C before use.

Sample treatment
The rat plasma samples at -40^{o}C were thawed under room temperature. Trichloroacetic acid (60μL 10%) and plasma sample (100μL) were mixed in a 1.5 mL centrifuge tube. After vortexed for 1 min, the mixture was placed at 4^{o}C for 30 min. The mixture was then centrifuged at 10,000 rpm for 5 min. The supernatant was transferred to another tube and 1ml of absolute ethyl alcohol was added. The samples were placed at 4^{o}C for 12h and centrifuged at 10,000 rpm for 5 min. The precipitation was collected and

dissolved with 100 µL ultra-pure water. A volume of 20 µL of sample was injected into the HPLC system with fluorescence detection for analysis.

Chromatographic conditions

HPLC (high performance liquid chromatograph): Agilent1100, Agilent, USA: Injection volume: 20 µL; Mobile phase: ultrapure water: 1 M NaCl = 85:15; Flow rate: 1 mL/min; Column (Agilent, USA): TSK-G4000SW (7.8 mm × 300 mm, 5 µm); Fluorescence detector (LS45, Perkinelmer, USA): Excitation wavelength Ex = 492 nm, emission wavelength Em = 540 nm.

Method validation

Verification of fucoidan –FITC experiment

Fucoidan– FITC experimental verification by fluorescence spectrophotometry

The same concentration of unlabelled fucoidan solution, FITC solution, mixed solution of FITC and fucoidan and FITC-fucoidan solution were detected by fluorescence spectrophotometry. The emission wavelength and the excitation wavelength slit width of the fluorescence spectrophotometer were set to 5 nm and the excitation wavelength was 492 nm. The fluorescence spectra of the sample solution were scanned, and the difference of the emission spectra of each sample was examined.

Fucoidan – FITC experimental verification by HPLC with fluorescence detector

The peak position and fluorescence intensity of fucoidan before and after FITC labelling were detected using chromatographic conditions described under section 2.5 to determine whether the marker successfully labelled the product.

Linearity of standard curve

The Quality control (QC) plasma samples of FITC-fucoidan were treated according to method described under section 2.4.

X-coordinate (x): the concentration of the quality control (QC) samples;

Y-coordinate (y): the peak area ratios (fucoidan/IS)

LLOQ and LLOD

A series of stepwise dilution was prepared from 1 mg/mL standard plasma sample of FITC-fucoidan diluted with blank plasma and added 0.1 mL dextran stock solution.

The lower limit of quantification (LLOQ) was the lowest concentration of plasma sample in the standard curve giving an acceptable accuracy (relative error, RE %) within ± 20% and a precision (relative standard deviation, R.S.D. %) that did not exceed 20%, which was ten-times of signal to noise ratio.

The LLOD was the lowest detectable plasma sample concentration in the standard curve, which was three-times the signal to noise ratio.

Intra-day and inter-day precision

Three different volumes 0.002 mL, 0.02 mL,0.08 mL of standard plasma sample of FITC-fucoidan were prepared and 0.01 mL dextran stock solution was added to each volume, and then diluted them with blank plasma to 1ml. Each concentration has 3 parallel groups.

Intra-day precision was tested by measuring 3 different concentrations of the QC samples for 5 replicates. Inter-day precision was tested by measuring 3 different concentrations of the QC samples in 5 consecutive days. Intra-day and Inter-day precision were presented by relative standard deviation (RSD). The precision was required to not exceed 15%.

Accuracy

Three different volumes 0.002 mL、0.02 mL、0.08 mL measured from standard plasma sample of FITC-fucoidan and added 0.01 mL dextran stock solution to each volume, and then diluted them with blank plasma to 1ml. Each concentration has 3 parallel groups.

Accuracy was measured by analysing 3 different concentrations of the QC samples for 3 replicates. The testing accuracy was showed as (nominal/observed concentration)×100% and the accuracy was required to be between 85 and 115%.

Stability

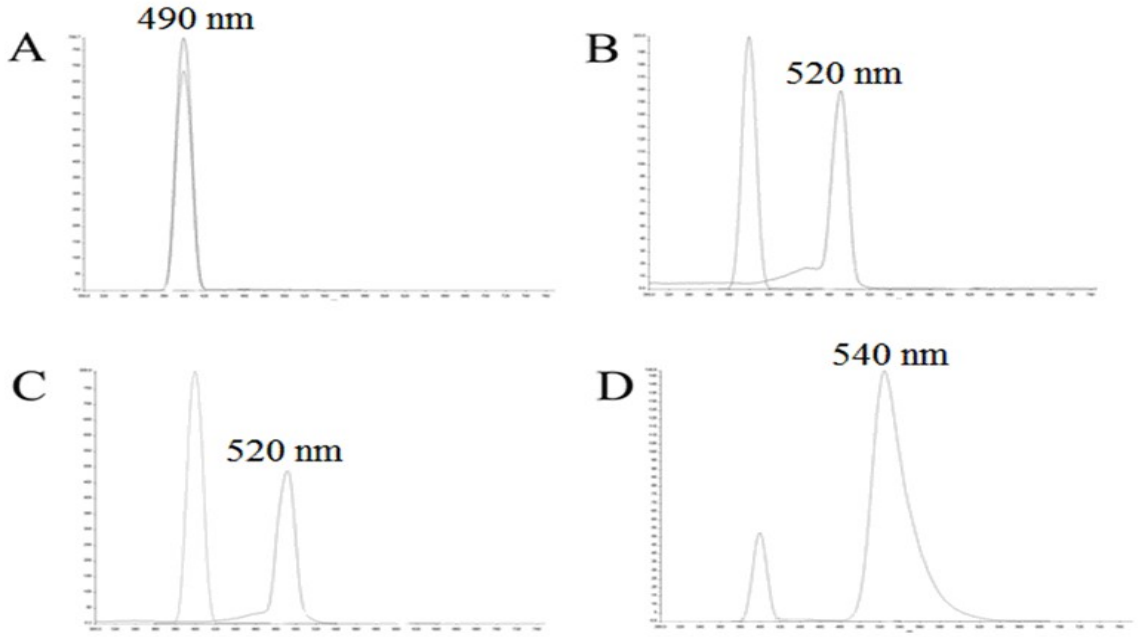
Three different volumes 0.002 mL' 0.02 mL' 0.08 mL measured from standard samples of FITC-fucoidan and added 0.01 mL dextran stock solution to each volume, and then diluted them with blank plasma to 1ml. Each concentration has 3 parallel groups. All samples were stored at different conditions: following three freeze (-40^{o}C)-thaw cycles; room temperature for 12 h; stored at 4^{o}C for 48 h; stored at -40^{o}C for 30 days.

Stability was tested by analysing 3 different concentrations of the QC samples for5 replicates. The stability was represented as (nominal/observed concentration) × 100%. The stability was required to be between85and 115%.

Extraction recovery and matrix effect

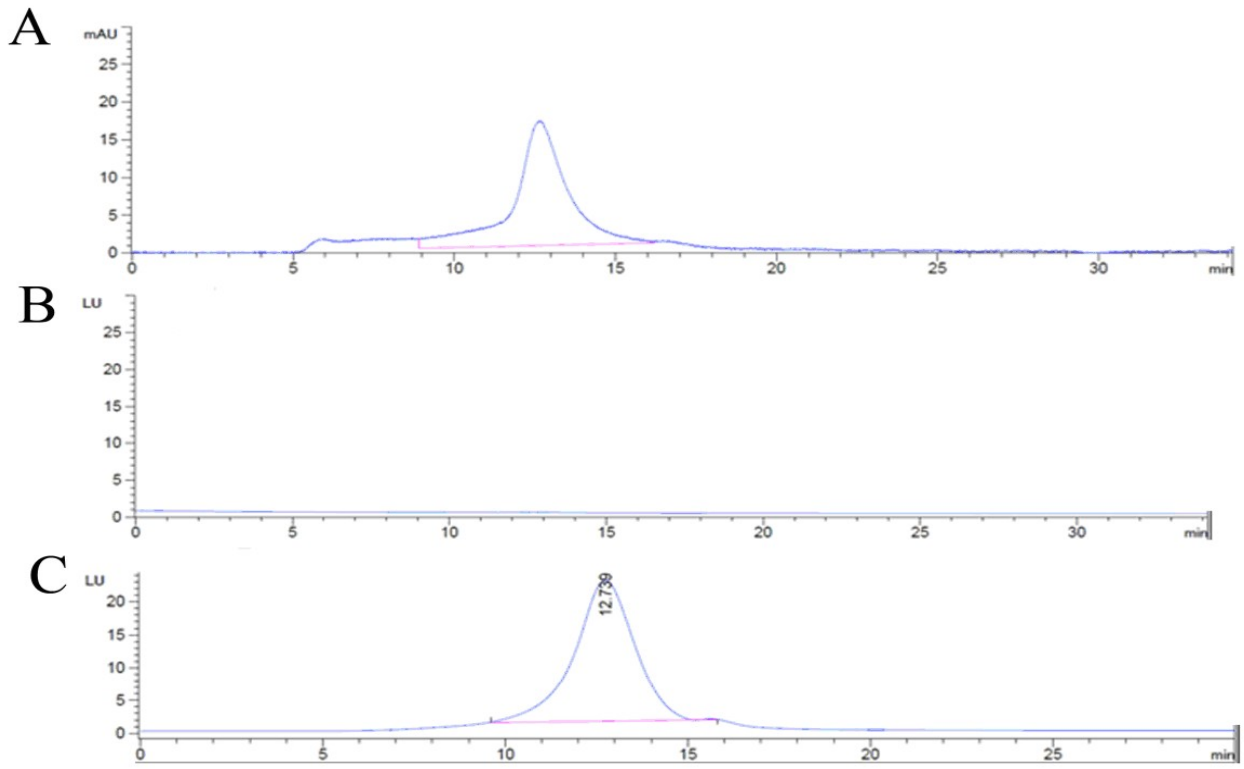
The chromatographic peak area A1: Three different volumes 0.002 mL' 0.02 mL' 0.08 mL measured from standard samples of FITC-fucoidan, and diluted them with blank plasma to 1ml. Each concentration has 3 parallel groups. Each sample have an according chromatographic peak area A1.

The chromatographic peak area A2. The blank plasma sample was treated according to 2.4. Sample treatments and then FITC-fucoidan was added to the final concentration of 20, 40, 80µg/Ml. Each sample has an according chromatographic peak area A2.



(A) Fucoidan solution; (B) FITC solution; (C) Fucoidan and FITC mixed solution; (D) FITC labelling fucoidan solution

Fig. 2: Fluorescence labelling fucoidan was tested by fluorescence spectrophotometry



(A) Fucoidan was tested by HPLC with a UV detector; (B) Fucoidan was tested by HPLC with a fluorescence detector;
(C) FITC labelling fucoidan was tested by HPLC with a fluorescence detector.

Fig. 3: Fluorescence labelling fucoidan was tested by HPLC with UV detector and fluorescence detector

The extraction recovery of fucoidan in rat plasma =A1/A2*100%.

The chromatographic peak area B1: The blank plasma sample was treated according to 2.4. Sample treatments and then FITC-fucoidan was added to the final concentration of 20, 40, 80μg/ml. All samples were filtered through 0.45μm filter and detected under the 2.6. HPLC conditions for detection. Each sample have an according chromatographic peak area B1.

The chromatographic peak area B2: The FITC-fucoidan was dissolved in 0.15 M NaCl to the final concentration of 20, 40, 80μg/ml. All samples were filtered through 0.45μm filter and detected under the 2.6. HPLC conditions for detection. Each sample have an according chromatographic peak area B2.
The matrix effect =B1/B2*100%

Specificity
The specificity was obtained from testing the following 4 samples: blank plasma; blank plasma included IS; blank plasma included FITC-fucoidan and IS; plasma samples from rat fed by FITC-fucoidan. The samples were detected under the 2.6. HPLC conditions for detection.

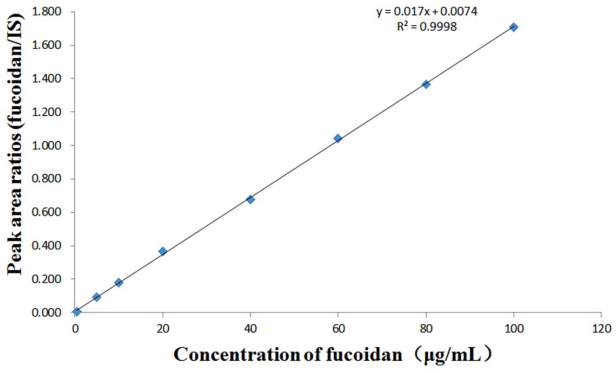


Fig. 4: Linear standard curve for fucoidan in plasma

Pharmacokinetic studies
The rats were laboratory fed under 20-24 °C and a 12 h/12 h light/dark cycle with food and water freely. The rats were in abrosia for 12h before the procedure, but could access to water throughout the experiment. FITC-fucoidan dissolved in ultra-pure water was administered intravenously at 6 mg/kg and orally at 20 mg/kg to 6 rats respectively. Blood samples (0.3mL) were serially withdrawn from the caudal vein into anticoagulant tubes with 0.45 mg EDTA at 0, 0.5,1, 2, 3, 4, 6, 12and 24 h after dosing. Then centrifuged the blood samples at 3000 rpm, 10 min after the blood samples (0.3mL) were serially withdrawn from the caudal vein into anticoagulant tubes and stored the supernatant plasma samples into another tube at -40^{o}Cfor analysis. All samples were detected under the 2.6. HPLC conditions for detection.

STATISTICAL ANALYSIS

The mean ± S.D was calculated by Microsoft Office Excel 2016. PK Solver 2.0 was used to analyse the pharmacokinetic parameters of the experiment. The pharmacokinetic parameters were analysed by non-compartmental model.

The maximum plasma concentration C_{max} and the time required to reach the C_{max} concentration T_{max} were measured values of concentration–time curve. For the plasma concentration - time curve, the linear regression was performed on the end points with the least square method. The slope of the regression line was the terminal phase elimination rate constant $k_{el.}$ The half-life ($t_{1/2}$)= $0.693/k_{el}$. The AUC_{0-t}(the area under the plasma concentration–time curve from time point 0 to the last measurable plasma concentration time point t) was calculated using the trapezoidal method. The $AUC_{0-\infty}$ (the area under the plasma concentration–time curve from time point 0 to∞) was the sum of AUC_{0-t} and $C_t/k_{el.}C_t$ was the plasma concentration corresponding to the last time point t. The MRT (the mean residence time)=$AUMC_{0-\infty}/AUC_{0-\infty}$.The$AUMC_{0-\infty}$ was the area under the first moment-time curve. The clearance rate $CL=(C_0)_{iv}/(AUC_{0-\infty})_{iv}$. The oral clearance rate $CL/F=D_{oral}/(AUC_{0-\infty})_{oral}$. D_{oral} was the oral fucoidan dose). Bioavailability $F=(D_{iv}\cdot AUC_{oral})/ (D_{oral}\cdot AUC_{iv})$. D_{iv} was the intravenous administered dose. D_{oral} was the oral fucoidan dose. The data were showed as mean ± standard deviation.

RESULTS

Method validation
Verification of fucoidan FITC experiment
Fucoidan FITC experimental verification by fluorescence spectrophotometer
The unlabelled fucoidan solution, FITC solution, mixed solution of FITC and fucoidan and FITC-fucoidan solution were measured by fluorescence spectrophotometry. The results are shown in fig.2.

The figure showed that the unlabelled fucoidan solution did not show a chromatographic peak at 520 nm, whereas the mixed solution of FITC and fucoidan showed the same chromatographic peak as the FITC solution at 520nm.The chromatographic peak at 540 nm of Fucoidan–FITC showed that FITC and fucoidan were successfully combined, and the chromatographic peak position produced a certain distance of Stoke's shift (red-shift). The results showed that FITC and fucoidan were successfully combined.

Fucoidan–FITC experimental verification by HPLC with fluorescence detector
The peak position and fluorescence intensity of fucoidan before and after FITC labelling were measured by HPLC.

The results are shown in fig3. Unlabelled fucoidan has no fluorescent peaks and no chromatographic peaks in the fluorescence detector, whereas the Fucoidan–FITC sample can detect the same chromatographic peak as that of unmarked fucoidan in the UV detector. These results indicate that the fucoidan was labelled by FITC.

Linearity of standard curve, LLOQ and LLOD

Linearity of the standard curve is very important for determination of biological samples. Linearity of the standard curve (fig. 4) was obtained by simple linear regression in the given concentration ranges of fucoidan in plasma. The correlation coefficient of calibration curves for fucoidan was 0.9998 and it showed good linearity between0.5～100μg/ml.

The LLOQ in plasma was measured by analysing different levels of concentration ranging from 0.5～20 μg/mL and it was measured to be 0.5μg/mL with 15.6% precision, within the acceptable criteria of less than

Table 1: Accuracy and precision for the analysis of fucoidan in QC samples (n=5).

Spiked concentration (μg/mL)	Precision (%)		Accuracy (%)
	Intra-day	Inter-day	
2	7.34	12.58	96.83±4.31
20	8.58	9.31	100.03±7.86
80	6.21	7.03	97.25±4.16

Table 2: Stability data of fucoidan in plasma QC samples under various condition (n=5).

Stability test	Spiked concentration (μg/mL)	Accuracy（%）
Short-term (room temperature for 12 h)	2	97.32±4.58
	20	98.81±5.83
	80	95.28±5.34
Post-preparative (at 4℃ for 48 h)	2	93.28±4.67
	20	95.73±6.81
	80	95.62±3.97
Long-term (at -40℃ for 30 days)	2	98.64±3.92
	20	92.76±5.27
	80	97.59±4.63
Three freeze (-40℃)-thaw cycles	2	96.32±4.73
	20	94.81±3.96
	80	97.88±5.41

Table 3: Recovery and matrix effect for the analysis of fucoidan in plasma QC samples (n=5).

Spiked concentration (μg/mL)	Recovery (%)	Matrix effect (%)
2	94.50±6.95	92.67±3.96
20	93.33±6.37	95.83±5.47
80	96.53±4.49	95.78±5.81

Table 4: Main pharmacokinetic parameters for fucoidan in rat plasma following intravenous administration (6 mg/kg) and oral administration (20 mg/kg) (mean ± SD, n=6).

Parameter	Unit	Administration method	
		i.v.	i.g.
C_{max}	μg/mL	75.59±16.74	7.33±1.38
T_{max}	h	0	2±0.53
k_{el}	1/h	0.173±0.06	0.169±0.04
$t_{1/2}$	h	4.01±0.97	4.10±0.84
AUC_{0-t}	g/mL*h	479.07±48.64	42.69±13.27
$AUC_{0-\infty}$	μg/mL*h	486.84±67.58	43.31±18.61
CL	(mg)/(μg/mL)/h	0.0037±0.0015	-
CL/F	(mg)/(μg/mL)/h	-	0.138±0.038
$MRT_{0-\infty}$	h	5.48±1.78	5.30±1.65
F	-	8.91%	

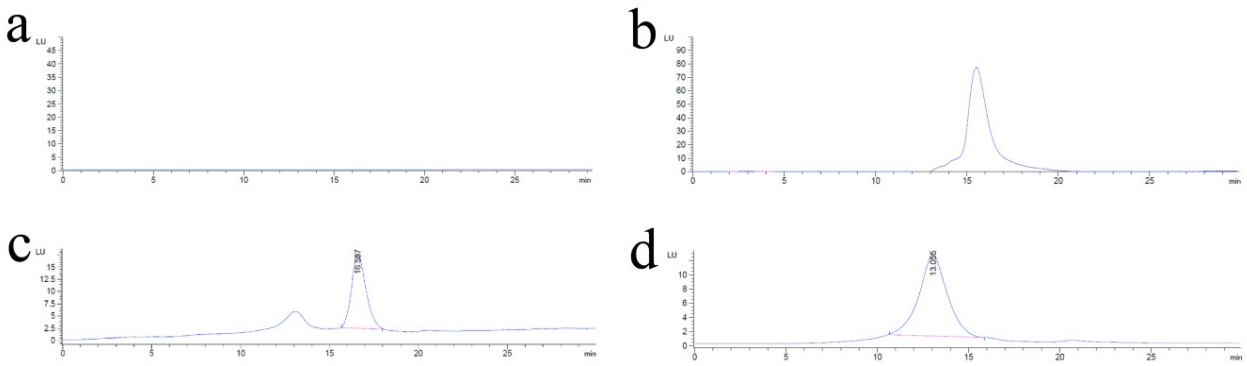


Fig. 5: Representative chromatographs of (a) blank plasma, (b) blank plasma contained IS, (c) blank plasma contained FITC-fucoidan and IS, and (d) plasma samples from rat fed by FITC-fucoidan

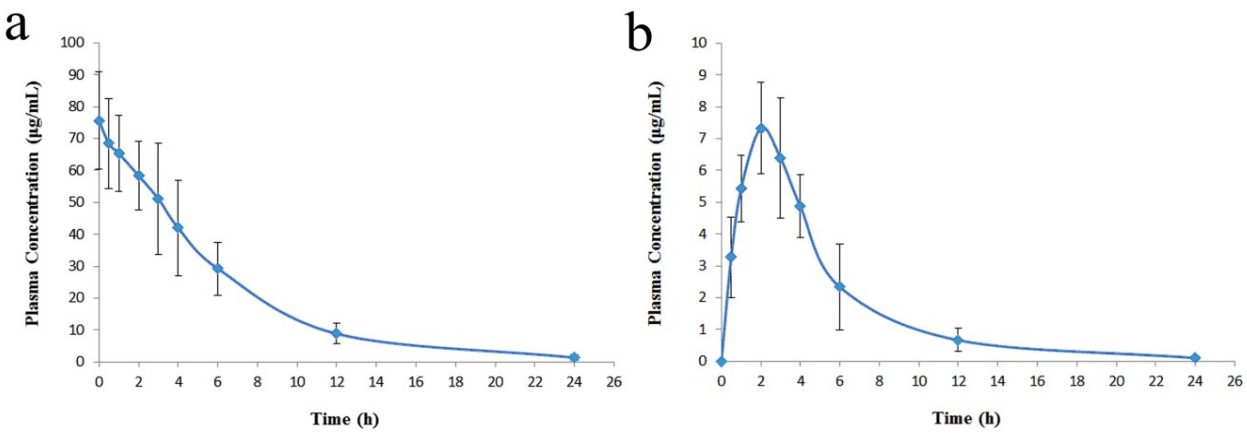


Fig. 6: Concentration-time curve for plasma samples after (a) intravenous administration and (b) oral administration

20%.The LLOD was measured to be 0.15μg/mL.

Precision and accuracy

Intra-day precision, inter-day precision and accuracy data are showed in table 1. The RSDs of the samples were tested at three QC concentration levels, ranging from 6.21 to 12.58% within the acceptable criteria of less than 15%. The accuracy was tested at 3different QC concentrations ranging from 96.83 to 100.03% within the acceptable criteria.

Stability

The stability of fucoidan in rat plasma under 3 conditions are listed in table 2. Fucoidan in rat plasma was steady in plasma samples after three freeze (-40°C)-thaw cycles, with samples stored under room temperature for 12 h, with samples stored at 4°C for 48 h, and samples stored at -40°C for 30 days.

Extraction recovery and matrix effect

The results of extraction recovery and matrix effect in rat plasma for fucoidan are listed in table 3. The extraction recovery ranges from 93.33 to 96.53% within the acceptable criteria. The matrix effect ranges from 92.67-95.83%. It suggests that no significant influence exits in this HPLC with fluorescence detection. Components in plasma were commonly not provided with fluorescence, so high-performance liquid chromatography with fluorescence detection using FITC-dextran as IS can reduce the matrix effect.

Specificity

The specificity was from the chromatograms of the following 4 samples: blank plasma; blank plasma contained IS; blank plasma contained FITC-fucoidan and IS; plasma samples from rat fed by FITC-fucoidan. The result shows in fig. 5, the retention times of FITC-fucoidan and IS were about 13.05 and 16.59 min each one. No interfering peak from endogenous substances can be observed at the retention time of fucoidan and IS.

Pharmacokinetic studies

The mean plasma concentration–time profiles of intravenous and orally administered fucoidan is presented in fig. 6a and fig. 6b, respectively. The major pharmacokinetic parameters of fucoidan are shown in table 4. The maximum fucoidan concentrations (7.33 μg/mL) was observed 2 h after oral administration. The CL of fucoidan after intravenous administration was 0.0037 (mg)/(μg/mL)/h and after oral administration was

0.138 (mg)/(μg/mL)/h. It showed that the fucoidan can be absorbed rapidly by the body after oral administration and is eliminated slowly.

DISCUSSION

Fucoidan can be successfully labeled by FITC with the aid of HPLC without any effect on fucoidan. It perhaps makes it more precisely and conveniently to detect the fucoidan in vitro and vivo.

Fucoidan as a high molecular weight polysaccharide can cross the intestinal barrier to blood circulation via two parallel routes: Transcellular and paracellular. It demonstrated that the permeation transportation of the high molecular weight fucoidan was restricted because of tight junctions and and might be transported by a carrier or endocytosis. The bioavailability of fucoidan from *Laminaria japonica* was 8.91% but was a high bioavailability exceeding the fucoidan from *Cladosiphon okamuranus,* which was transported across Caco-2 cells at a bioavailability of less than 0.01% (Nagamine *et al*., 2016). The fucoidan from *Laminaria japonica* is of lower molecular weight than the fucoidan from *Cladosiphon okamuranus*, so it can be more easily absorbed by the intestinal tract, resulting in a better bioavailability. The advantages of fucoidan from *Laminaria japonica* can be used to develop a potential source for therapeutic agents. Further detailed studies to elucidate the absorption mechanism and pharmacokinetics need to be performed.

It has always been an obstacle to detect the fucoidan in vitro an vivo. This paper establishes an accurate and convenient way to detect the fucoidan. With the help of this method we can further to study the distribution of fucoidan in other organs.

CONCLUSIONS

A new expanded method of determining the characterisation of fucoidan in rat plasma through HPLC with fluorescence detection was improved and verified and which was applied to the study of pharmacokinetics after the oral administration and Intravenous injection. In rats, the fucoidan from *Laminaria japonica* can be absorbed rapidly by the body after feeding and is eliminated slowly. Fucoidan from *Laminaria japonica* exhibited bioavailability superior to that of fucoidan from *Cladosiphon okamuranus*, indicating that *L. japonica* presents a superior source for potential therapeutic agents.

Compliance with ethical standard

The use of experimental animals in this study was conducted in accordance with the ethical guidelines of the Shandong University animal experimentation committee.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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