Development and validation of an LC-MS/MS method for the quantification of flavonoid glucuronides (wogonoside, baicalin, and apigenin-glucuronide) in the bile and blood samples: Application to a portal vein infusion study

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Abstract

Glucuronidation is one of the major metabolic pathways for flavonoids. However, quantification of flavonoid glucuronides in biological samples, especially in the bile, is sometimes challenging due to signal suppression by bile acids. The purpose of this study is to establish a robust LC-MS/MS method for directly measuring flavonoid glucuronides in bile and blood. Wogonoside (wogonin-7-O-glucuronide), baicalin (baicalein-7-O-glucuronide) and apigenin-7-O-glucuronide were used as the model compounds and taurocholic acid (T-CA) were used as the model bile acid to establish the method. Bile samples were processed using solid phase extraction (SPE) and blood samples were prepared using protein precipitation method. The analytes were separated on a Resteck HPLC (50 mm × 2.1 mm ID, 1.7 μm) column using acetonitrile and 0.1% formic acid in water as the mobile phases. The mass analysis was performed in an AB Sciex 5500 Qtrap mass spectrometer via multiple reaction monitoring (MRM) in the positive mode. The results showed that the linear range of the above three analytes were 10 nM–5000 nM in the bile and 1.56 nM–4000 nM in the blood, respectively. The recoveries of three glucuronides were> 85% and the matrix effects were <20% at low, medium and high concentrations in the bile and the blood. The results also showed that >90% of these bile acids were removed by the selected SPE procedure to facilitate glucuronide analysis. The validated method was successfully applied to a portal vein infusion study using rats to quantify baicalin, wogonoside, and apigenin-glucuronide in bile and blood samples.

1. Introduction

Flavonoids, which consists of two phenyl rings and a heterocyclic ring in the structures, are widely distributed in many fruits and vegetables [1]. For example, apigenin, a 5,7,4′-trihydroxyl-flavone, is found as one of the major ingredients in chamomile tea and the contents of apigenin is up to 0.8%–1.2% in a typical chamomile tea drink [2]. Other examples include genistein in soy, quercetin in oranges, and anthocyanidins in blueberries [3,4]. Flavonoids possess multiple pharmacological effects, including anti-cancer, anti-inflammation, and anti-oxidation [5–7], probably due to their anti-oxidation property from hydroxyls. Therefore, this class of compounds is believed to be the active components in nutritional and herbal materials. For example, wogonin was reported as the key active components against inflammation in *Scutellaria Radix* [8].

Although bio-activities of flavonoids are promising, their oral bioavailabilities are usually poor [9,10], which hampers flavonoids being developed as therapeutic drugs. Pharmaceutical research has demonstrated that one of the major reasons for poor oral bioavailability for flavonoids is glucuronidation in the intestine and the liver [11,12]. Interestingly, some flavonoid-glucuronides are good substrates of certain efflux transporters (e.g., BCRP, MRP2) [13] and can be secreted through bile into the intestine, where glucuronides can be hydrolyzed by microflora to release the aglycones, followed by re-absorption in the intestine to form a enterohepatic recycling (EHR) [11,12]. Many studies have been conducted to study enterohepatic recycling in order to have a better understanding and utilization of this physiological phenomenon. Thus, sensitive and robust analytical method to quantify flavonoids and their glucuronides in the bile and blood samples are required.

A few LC-MS methods for flavonoid-glucuronides quantification in the bile and blood have been published previously [14–20]. However, these methods are not widely cited probably because of low sensitivity and complex sample preparation procedures. Further studies suggested that low signal sensitivity in the bile might be caused by ion suppression from bile acids [21]. In addition, bile acids can form micelles and encapsulate the analytes, resulting in low extraction recovery [22,23]. Alternatively, a common approach to analyze glucuronides in the bile is to hydrolyze the conjugates and quantify the aglycones [24,25]. However, this indirect quantification method is not robust and may not be accurate because stability is a concern for some flavonoids during hydrolysis. Moreover, this indirect quantification can't quantify both glucuronides and aglycones at the same time, which is required in many studies.

This paper developed and validated a LC-MS method for glucuronides quantification in the bile and blood samples and apply the method in a portal vein infusion study, where both bile and blood samples are collected.

2. Experiments

2.1. Chemicals and reagents

Wogonoside was purchased from Meilunebio (Dalian, China). Apigenin-7-O-glucuronide was purchased from HWI Analytik GmbH (Rheinzaberner, Rülzheim, German). Rutin (internal standard, I.S.) was purchased from Cayman Chemistry Company. Baicalin was purchased from Indofine (NJ, USA). DMSO was purchased from Sigma-Aldrich. Acetonitrile and methanol were from Omni Solv (CA, US). HLB cartridge and MCX cartridge were purchased from Waters Oasis (MA, USA). X-AW, C18–W and SCX cartridge were purchased from Strata Phenomenex (CA, USA.). Other chemicals were used as received.

2.2. Instrument and conditions

2.2.1. UPLC conditions—UPLC condition: system, Waters Acquity with diode array; column, Restek Ultra BPh (5 μm, 100 mm × 2.1 mm); mobile phase, A 0.1% formic acid in water, mobile phase B acetonitrile; gradient, 10% B (0–0.5 min), 10% B–34% B (0.5–1.0 min), 34% B–60% B (1.0–2.5 min), 60% B–95% B (2.5–6.0 min), 95% B–10% B (6.0–7.0 min); flow rate, 0.45 ml/min; column temperature, 45 °C; injection volume, 10 μL.

2.2.2. Mass spectrometry conditions—The MS analysis was performed on a Sciex 5500 triple quadrupole mass spectrometer (AB Sciex LLC, Framingham, MA) equipped with an ESI source. The detection was conducted using MRM scan type in positive ion mode. The instrument dependent parameters were: ionspray voltage, 5.5 kV; ion source temperature, 400 °C; nebulizer gas (gas 1), nitrogen, 20 psi; turbo gas (gas 2), nitrogen 20 psi; curtain gas, nitrogen 20 psi. Unit mass resolution was set in both mass-resolving quadruples Q1 and Q3. Compound-dependent parameters were listed in Table 1.

2.3. Preparation of stock solution, calibration curves, and QC samples in the bile and blood

Stock solutions (1.0 mM) of wogonoside, baicalin, apigenin-7-O-glucuronide, and rutin were prepared in water:acetonitrile = 1:1 (containing 0.1% formic acid). The working solution for bile samples preparation were prepared by serial dilution of the stock solution into 50% methanol to afford 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 μM, respectively. To prepare calibration curve samples in the bile, these working solutions (5 μL each) were spiked into 5 μL of blank bile, after which the mixtures were further diluted in 1 mL of water (containing 0.1 μM rutin as internal standard)and loaded to SPE columns. After eluted from the SPE column with elution solutions described below, samples were collected, and solvents were removed under N_2 flow. The residues were reconstituted into 20% acetonitrile (200 μL) for LC-MS analysis to reach a final concentration of 5,000, 2,500, 1,250, 625, 312.5, 156.3, 78, 39, 20, 10 nM. The quality control (QC) samples for each analyte were prepared at three different concentrations (10.0 nM as low, 156.25 nM as medium, and 2500.0 nM as high) following the same procedures as described above. Standard curves were prepared every time when a new batch of samples were prepared and injected with samples.

The working solution for blood samples preparation were prepared by serial dilution of the stock solution into 50% methanol to afford 4,000, 2,000, 1,000, 500, 250, 125, 62.5, 31.2, 15.6 nM, respectively. To prepare standard curve in the blood, blank blood (10 μL) was mixed with 10 μL of standard curve solutions. Then, the mixture was further added with 200 μL of acetonitrile (containing 100 nM rutin as internal standard). The mixture was further vortex for 1min and centrifuged at 15,000 rpm for 15min. Then, 200 μL of supernatant was collected and dried under nitrogen flow. The residues were reconstituted into 20% acetonitrile (200 μL) to reach a final concentration of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 nM for LC-MS analysis. The quality control (QC) samples for each analyte were prepared at three different concentrations (1.56 nM as low, 25.0 nM as medium, and 200.0 nM as high) following the same procedures as described above.

2.4. Sample preparation

Bile samples preparation.—The bile samples were prepared following the procedures described in the calibration curve samples preparation. Briefly, 5 μL of bile samples was spiked into 5 μL of 50% acetonitrile, which was further diluted into 1 mL of water for SPE extraction.

Blood samples preparation.—Blood samples were also prepared following the procedures described in the calibration curve samples preparation. Briefly, 10 μL of blood samples was spiked into 10 μL of 50% acetonitrile, which was further precipitated by 200 μL of Methanol (with 0.1% formic acid and IS).

2.5. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) of bile samples

To establish a reliable extraction method, wogonoside was selected as model compound and T-CA, the most abundant bile acid in rat bile [26], was selected as the marker of bile acid. Methanol, which is a typical solvent used for protein precipitation [27–29], was used in LLE. Briefly, 5 μL of QC samples at three concentrations (low, medium and high) were spiked with 5 μL of blank bile. Then, 1 mL of methanol (with 0.1% formic acid and IS) was applied to precipitate the bile acids and proteins. After vortex and sonicate for 15min, samples were further centrifuged at 15,000 rpm for 15min, after which 950 μL of supernatant was taken and dried under nitrogen flow. Then, the residue was reconstituted into 200 μL of 20% acetonitrile for LC-MS analysis.

To establish an appropriate SPE procedure, five commonly used SPE cartridges were tested. Briefly, wogonoside (5 μL) was mixed with blank bile (5 μL) and diluted to 1 mL of water with 0.1% formic acid at the concentration of 1 μM. Internal standard (rutin) was added in and diluted at 0.5 μM in the mixture. Then, samples were loaded onto the cartridges, which were eluted sequentially with 1 mL of water with 0.1%formic acid (loading fraction) and then 1 mL of methanol with 0.1% formic acid twice (MeOH elution). The collected fractions were dried under nitrogen flow and reconstituted with 200 μL of 20% acetonitrile for LC-MS analysis.

For elution optimization, different percentage of methanol applied in elution solutions were tested. Briefly, after sample loading, 30%, 40%, 50%, or 60% of methanol (1.0 mL) were

eluted respectively to afford the pre-elute fractions (Fr. 1). Then, 100% of MeOH was used to eluted to afford MeOH fraction (Fr. 2). After that, solvents were dried under nitrogen blowing and the residues were re-constituted into 20% of acetonitrile (200 μL) for LC-MS analysis.

2.6. Method validation

The analytical method was validated according to the "Guidance for Industry, Bioanalytical Method Validation" presented by the US Food and Drug Administration (2018) [30].

2.6.1. Specificity—The specificity of the method was determined by injecting processed pooled blank bile and pooled blank blood samples.

2.6.2. Linearity and lowest limit of quantification (LLOQ)—Calibration curves were prepared as described before. After sample analysis, a least-square linear regression ($1/X^2$ weights) was applied to determine the slope, intercept and correlation coefficient factor. The lowest limit of quantification (LLOQ) was determined accurately and precisely with a signal-noise ratio (S/N) of at least 5:1.

2.6.3. Recovery, matrix effect, and carry-over—The recovery was evaluated by comparing the peak areas obtained from samples prepared from blank bile/blood spiked with QC samples with those from samples prepared from water spiked with the same concentrations.

The matrix effect was determined by comparing the peak areas of samples prepared from residues of pooled bile/blood spiked with QC samples with those from residues of water spiked with the same concentration of QC samples.

The carryover impact was evaluated by inject blank matrix (50% acetonitrile, no analyte and IS.) right after the injection of the highest concentration of calibration standards.

2.6.4. Effect of T-CA on wogonoside's ionization—The effect of T-CA on wogonoside's ionization was evaluated by comparing the peak areas of QC samples at a low (156.2 nM) and a high concentration (2500 nM) spiked with T-CA (20 mg/mL) with those spiked with water.

2.6.5. Precision and accuracy—Precision and accuracy were conducted by replicate the analysis of QC samples (n = 3). The intra-day accuracy was conducted by performing the analysis in same day and 3-day accuracy was conducted by performing the analysis in consecutive 3 days.

2.7. Application to a portal vein infusion experiment

Animals.—The animal protocol used in this study was approved by University of Houston Institution of Animal Care and Use Committee. Male Wistar rats (270–320 g) were housed in an environmentally controlled room (temperature: 25 ± 2 °C, humidity: 50% ± 5%, 12 h day-night time cycle) at least a week before the experiment.

Portal vein infusion: The portal vein infusion study has been performed using a method was previously published by us [31]. Briefly, rats were fasted over-night with free access of water before the day of experiment. After a rat was anesthetized, its superior mesenteric vein in the mesentery of jejunum, which is directly connected to the portal vein, was exposed. The superior mesenteric vein was punctured by a venous indwelling needle. Then, rat's bile duct was located and separated from adjacent tissues. After that, a small cut was made with a micro vascular scissor and a tubing (PE10) was inserted and secured with a surgical suture. Its bile duct was also cannulated as described [31]. Drug solutions were infused from the portal vein cannulation and bile samples were collected from bile duct cannulation. The samples were collected every 0.5 h.

3. Results

3.1. Method development

Both UPLC and MS conditions were optimized. For LC condition, formic acid (0.1%) in water and acetonitrile were used as the mobile phases. For MS conditions, positive mode was selected for all analytes based on the method specificity and signal intensity comparing with negative mode. MRM scan type was used to increase the specificity of the method. Each compound was tuned and optimized separately to improve the analysis sensitivity. The parent ions and fragment ions with highest abundance were selected as the ion pairs for each analyte. The compound dependent parameters were summarized in Table 1. Rutin was selected as internal standard (I.S.) and added into the solvent used for extraction. Loss of the glucuronic acid unit (-176) is the major fragment ions for a glucuronide as the bond between the aglycone and the glucuronic acid is the easiest one to be broken in MS/MS. This fragment is commonly used in glucuronide quantification in LC-MS/MS in literature [32–34]. The MS/MS spectrum of 3 analytes were also presented in Fig. 1.

3.2. Liquid-liquid extraction (LLE) for bile sample preparation

The results of LLE were summarized in Table 2. The recovery and matrix effect were out of the acceptable ranges (85–115%), especially at low concentration, suggesting components in the bile suppressed ionization of the analytes. Protein precipitation using organic solvent was not an appropriate method for bile sample preparation.

3.3. Solid phase extraction (SPE) for bile sample preparation

First, the retention of wogonoside on the SPE cartridges were tested. The results showed that < 15%, which is the acceptable analytical error range, of the loading wogonoside was detected in the loading fraction when HLB cartridge used (Fig. 2A) and > 85% of wogonoside was in the MeOH elution (Fig. 2B). Based on this finding, HLB cartridge was selected as the SPE cartridge.

Then, the elution condition was optimized based on the wogonoside's recovery and its separation with T-CA in the elution. The results showed that when 40% of MeOH was used as the pre-elution solvent, TCA can be eluted entirely (> 85%, Fr. 1 in Fig. 3) and only tiny amount of T-CA (< 15%) was in the final elution (Fr. 2 in Fig. 3). While for wogonoside, 40% MeOH pre-elution couldn't elute wogonoside (< 15%, Fr. 1 in Fig. 3) and most of

wogonoside (> 85%) was in the final elution (Fr. 2, Fig. 3). These findings revealed that with 40% of MeOH pre-elution, wogonoside can be separated with T-CA on HLB cartridge. Therefore, of MeOH pre-elution will be used in the preparation of bile samples.

3.4. Effect of T-CA on wogonoside's ionization

The effect of bile acid on wogonoside's ionization was determined by spiking T-CA into QC sample. The spiked T-CA was 20 mg/mL, which is a concentration lower than the real bile samples but close to the solubility limit of T-CA in water [26,35]. The results showed that in the presence of T-CA, the intensity of wogonoside was decreased when compared to those spiked with water (Fig. 4). This result suggested that components in the bile (e.g., bile acid) suppress glucuronide's ionization. Therefore, remove bile acids in a key step in sample preparation in LC-MS analysis.

3.5. Method validation

3.5.1. Selectivity—The specificity was determined by comparing the samples at LLOQ with those from blank bile and blood samples pooled from six animals. As shown in Fig. 2, no significant interfering was observed with the analytes including baicalin, wogonoside and apigenin-7-O-glucuronide. The chromatographs where 3 analytes in bile and blood matrix were compared to corresponding blank matrix were summarized in Fig. 6. The comparison using second transitions was summarized in supplement Fig. S1.

3.5.2. Linearity, LLOQ, precision and accuracy—In the bile, the calibration curves were linear from 10.0 nM to 5000.0 nM for wogonoside, from 10.0 nM to 5000 nM for baicalin, and from 10.0 nM to 5000.0 nM for apigenin-7-O-glucuronide. The correlation coefficient (r^2) was at least 0.98 for these glucuronides (Table 3). The lower limit of quantification (LLOQ) for these 3 glucuronides are 10 nM. In the blood, calibration curves were linear from 1.56 nM to 4000.0 nM for wogonoside, from 1.56 nM to 4000.0 nM for baicalin, and from 1.56 nM to 4000.0 nM for apigenin-7-O-glucuronide. The correlation coefficient (r^2) was at least 0.99 for these glucuronides (Table 3). The lower limit of quantification (LLOQ) for these 3 glucuronides are 1.56 nM. The accuracies (%) of each concentration in standard curves were also summarized in Table 4. The intra-day and 3-day precisions and accuracies were summarized in Table 5. All the accuracies and precisions were within the acceptance values (100% ± 15%).

3.5.3. Matrix effect, recovery and carryover effects—In the bile, the extraction recoveries were evaluated using QC samples (n = 6) at low (10.0 nM), medium (156.2 nM) and high (2500 nM) concentrations. The mean recovery was over 95%, suggesting that SPE could extract these glucuronides from bile. The matrix effect after SPE preparations were within the range of 85%–105% for wogonoside, 95%–105% for baicalin, and 95%–105% for apiginen-7-O-glucuronide (Table 2).

In the blood, the matrix effect and recoveries in all three samples were all close to 100%, indicating that the method was good enough to analyze these glucuronides in blood samples without hydrolysis.

To determine carryover, blank matrix samples were injected right after the highest concentration of calibration standards. The results showed that the analytes and IS peaks detected in the blank matrix in both blood and bile are < 20% of LLOQ.

3.6. Application of the LC-MS method to analyze bile and blood samples derived from a portal vein infusion experiment in rats

The application of the established method was demonstrated by conducting rat portal vein infusion experiment. Wogonoside, baicalin and apigenin-7-O-glucuronide. The results showed that when the compounds were infused through the portal vein, the analytes can be detected in both the bile and blood (Fig. 5), suggesting that these three flavonoid glucuronides were taken up by the hepatocytes and then secreted to the apical side into the bile or basolateral side into the blood. Since glucuronides are highly hydrophilic, uptake transporter may be involved in the liver. Further studies need to focus on transporter identification to fully elucidate the mechanism. The recovery of wogonoside in the bile was significantly higher than the other two compounds, suggesting that the efficiency of enterohepatic recycling of wogonoside is better than the other two compounds. For baicalin, both biliary and blood exposure were very low, indicating that this compound might have a very fast clearance through urine.

4. Conclusion

In conclusion, a sensitive LC-MS method for directly analyzing flavonoid glucuronides in the bile and blood was established and validated. The method was applied in a portal vein infusion study. The current data suggested that bile acids suppress glucuronides ionization and SPE with HLB can efficiently separate flavonoid-glucuronides from bile acid in bile matrix. More studies are needed to further investigate the mechanism of enterohepatic recycling and clearance of these glucuronides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

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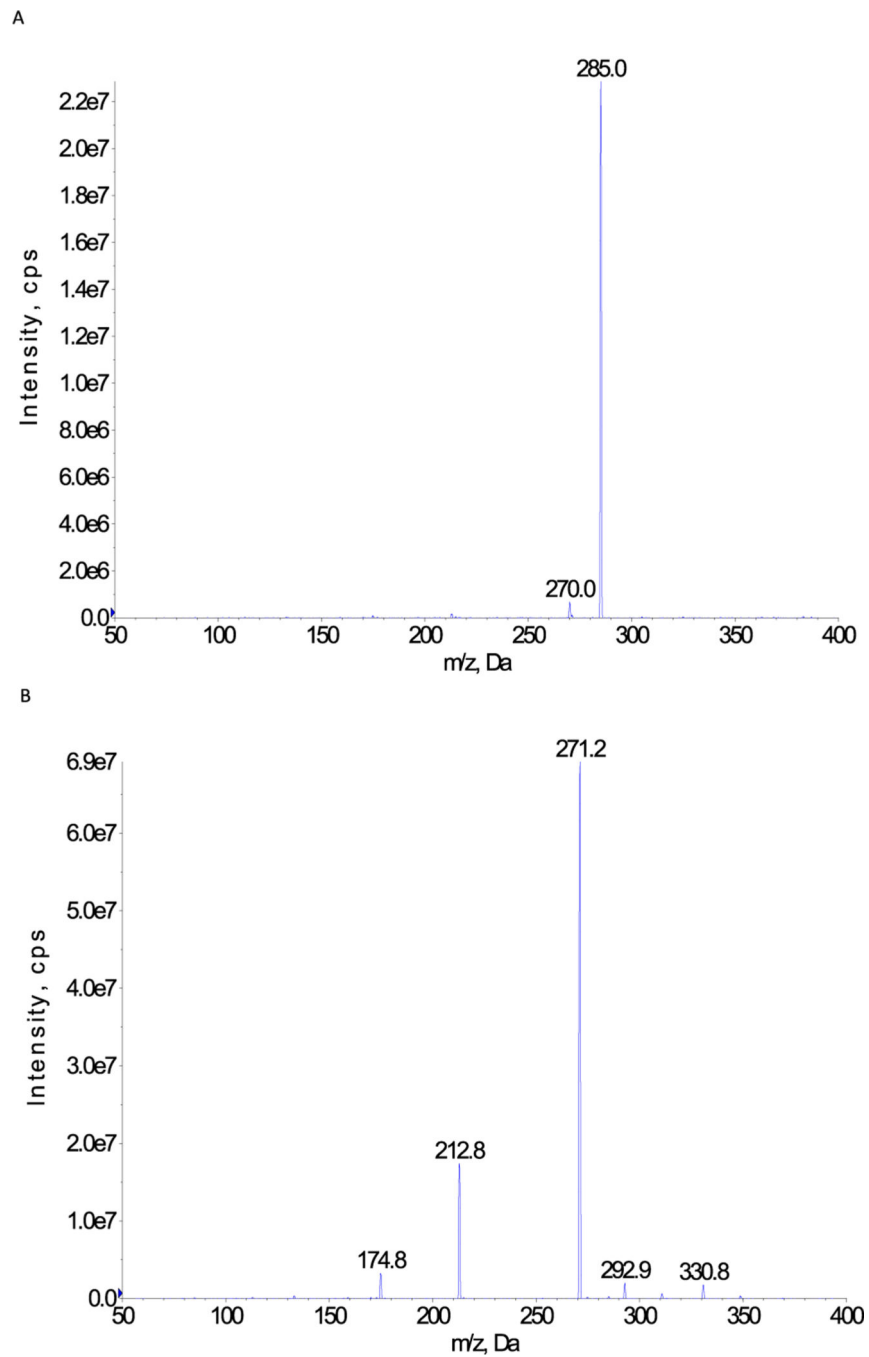
Fig. 1.
A The MS/MS spectrum of wogonoside (461.0) **B** The MS/MS spectrum of baicalin/apigenin-7-O-glucuronide (447.0).
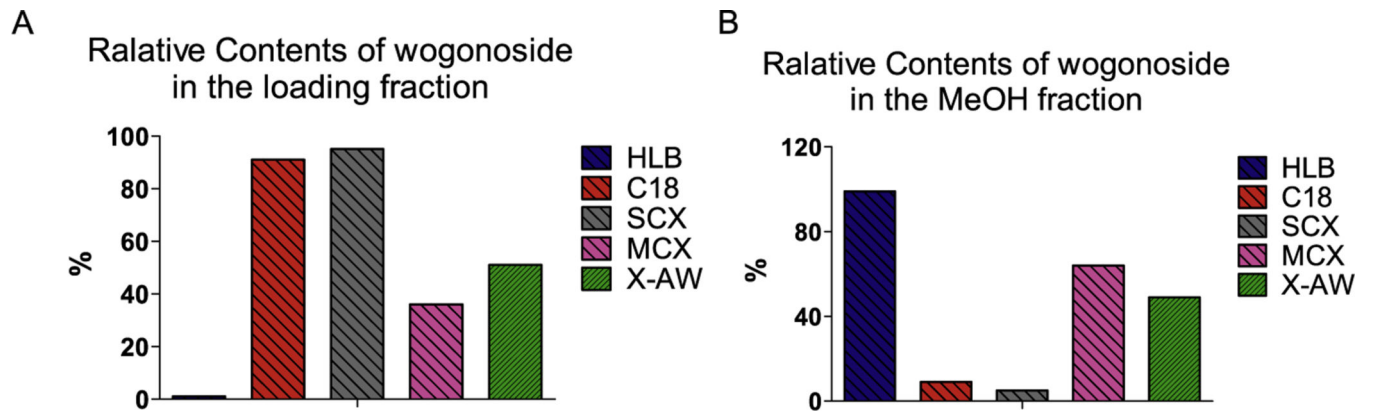
Fig. 2.
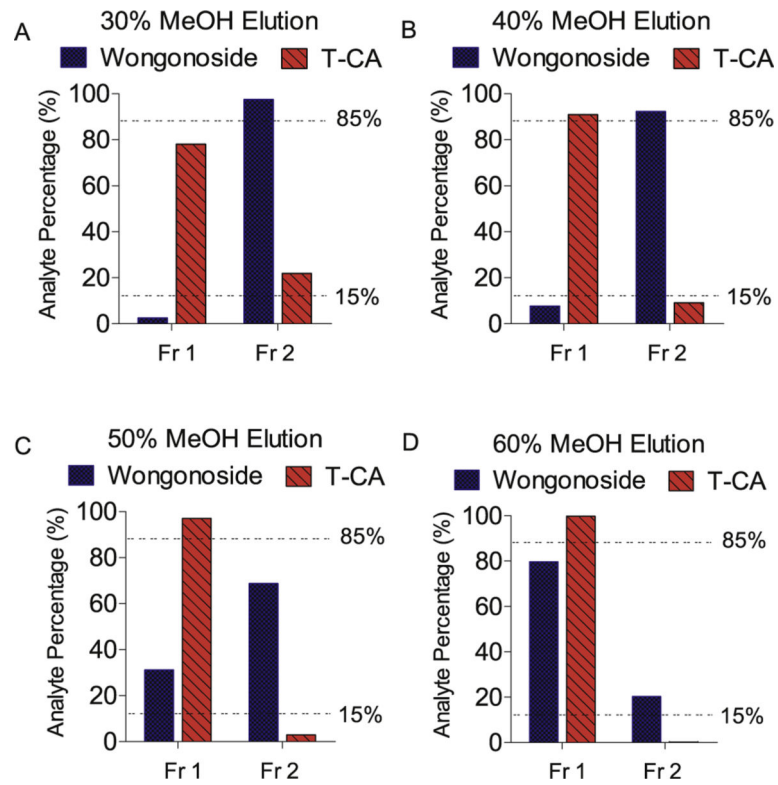The retention of wogonoside on different SPE cartridges.

Fig. 3.
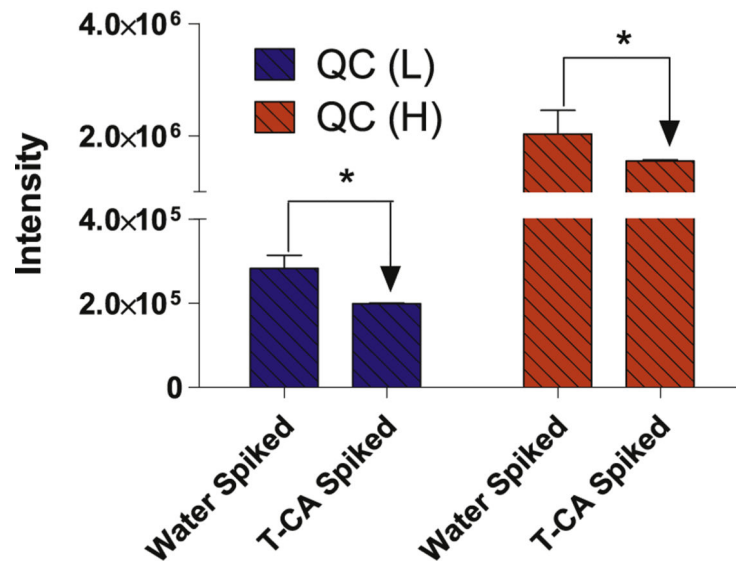Recovery and separation of wogonoside in SPE extraction with HLB cartridge. (Fr. 1, pre-eluted fraction, Fr. 2100% MeOH eluted fraction).

Fig. 4.
The impact of T-CA on wogonoside ionization. The addition of T-CA significantly decreased the signal intensity of wogonoside. Statistics were conducted by Student *t* test *p< 0.05.*
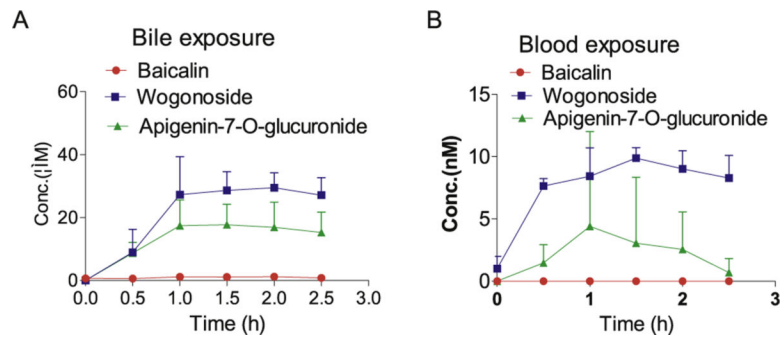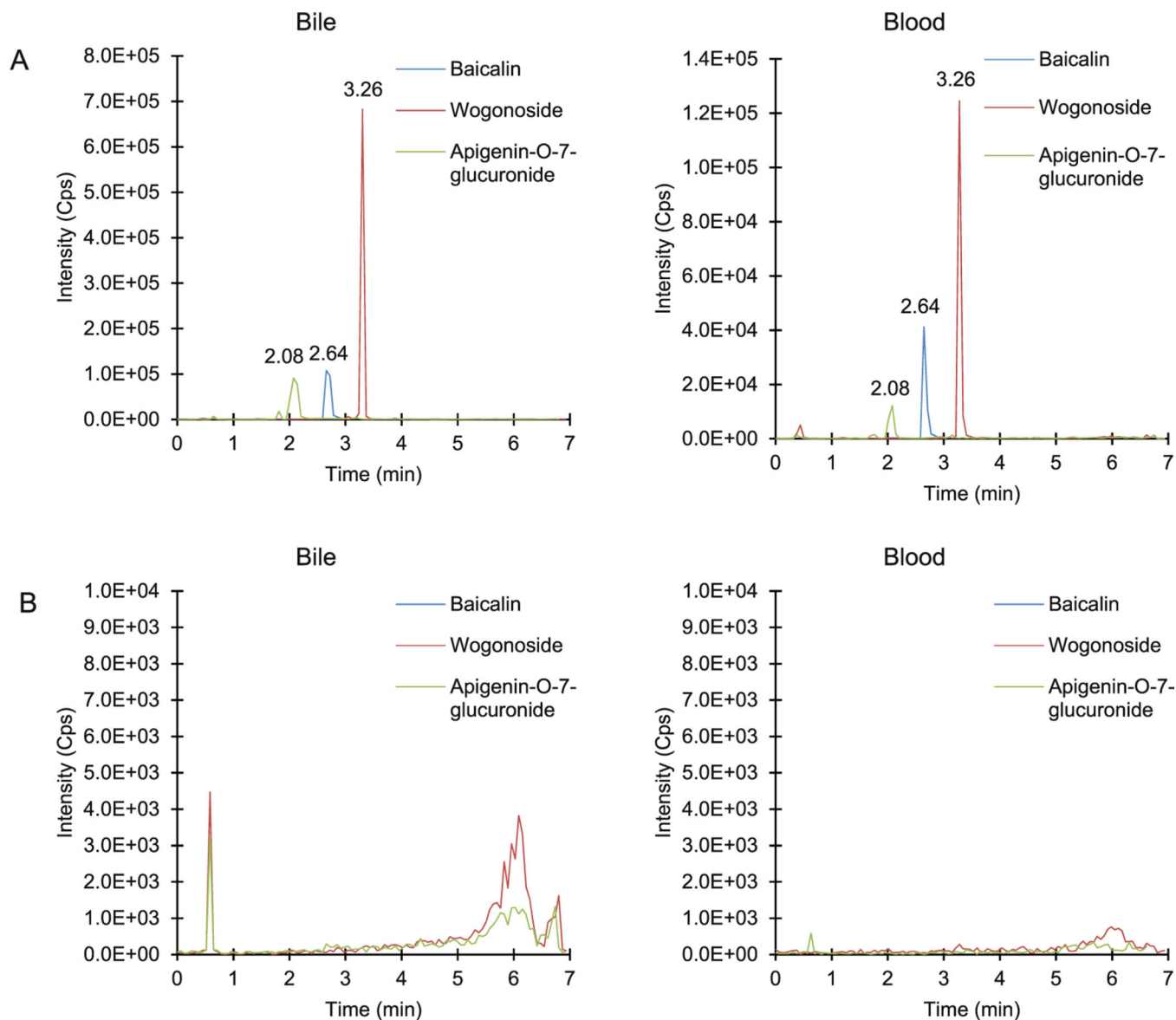
Fig. 5.
The concentrations of wogonoside, apigenin-7-O-glucuronide, and baicalin in the bile and blood in a portal vein infusion study.

Fig. 6.
A The LC/MS chromatographs of apigenin-7-O-glucuronide (green), baicalin (blue) and wogonoside (red) in bile and blood matrix. The retention time of each peak was marked in the chromatographs. **B** The LC/MS chromatographs of blank bile and blood matrix without any spiked analytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Compound dependent parameter of the analytes and I.S.

Compound	Q1/Q3	DP	CE	EP	CXP
Wogonoside[a]	461.0/285.0	30	26	10	16
Wogonoside[b]	461.0/270.0	34	21	10	11
Baicalin[a]	447.0/271.0	31	37	10	17
Baicalin[b]	447.0/175.0	36	28	10	12
Apigenin-7-O-glucuronide[a]	447.0/271.0	31	37	10	17
Apigenin-7-O-glucuronide[b]	447.0/175.0	36	28	10	12
T-CA	514.0/80.0	120	78	10	16
Rutin (I.S.)	611/303	120	47	10	13

[a] This ion pair was used for quantification for its highest signal intensity.

[b] This ion pair was used to verify the results in case of false positive results.

Table 2

The recovery and matrix effect of wogonoside in Liquid-liquid extraction in the bile.

Concentration	Recovery (%)	Matrix effect (%)
Low	ND	ND
Medium	70.6 ± 2.3	61.3 ± 5.2
High	76.7 ± 6.4	69.1 ± 1.3

ND: not detected.

Table 3

The linearity, LLOQ, and R^2 of wogonoside, apigenin-7-O-glucuronide and baicalin in the bile and blood. The accuracies (%) at each concentrations of the standard curves were presented.

Analytes	Linearity		LLOQ (nM)		R^2	
	Bile	Blood	Bile	Blood	Bile	Blood
Wogonoside	10.0–5000.0	1.56–4000.0	10.0	1.56	0.983	0.991
Apigenin-7-O-glucuronide	10.0–5000.0	1.56–4000.0	10.0	1.56	0.996	0.989
Baicalin	10.0–5000.0	1.56–4000.0	10.0	1.56	0.995	0.992

Conc.(nM)	Accuracy in Blood (%)		
	Wogonoside	Apigenin-O-7-glucuronide	Baicalin
1.56	92.7	98.2	112
3.12	111	135	81.1
6.25	112	118	90.7
12.5	86.1	93	102
25	112	111	94.3
50	88.1	77.2	109
100	98.7	91.4	102
200	101	100	110
400	98	85.1	100

Conc.(nM)	Accuracy in Bile (%)		
	Wogonoside	Apigenin-O-7-glucuronide	Baicalin
10	90.8	86.9	97.9
20	110	119	86.7
39	109	108	124
78	111	98.9	100
156	113	91	103
312	108	118	115
625	96.7	108	100
1250	98.1	107	96.9
2500	85.2	95.5	86.8
5000	79.5	80.9	86.6

Table 4

The recovery and matrix effect of wogonoside, apigenin-7-O-glucuronide and baicalin in bile and blood matrix.

Analytes	QC[a]	Recovery (%)		Matrix effect (%)	
		Bile	Blood	Bile	Blood
Wogonoside	Low	88.3 ± 13.2	99.8 ± 8.1	99.2 ± 12.4	103.1 ± 3.3
	Medium	100.5 ± 15.1	86.7 ± 7.5	98.4 ± 15.7	104.8 ± 8.2
	High	114.4 ± 1.3	87.3 ± 2.4	85.6 ± 3.7	102.1 ± 1.1
Apigenin-7-O-glucuronide	Low	88.2 ± 7.5	88.7 ± 7.2	103.6 ± 10.4	103.1 ± 3.9
	Medium	103.2 ± 5.2	90.7 ± 8.3	101.5 ± 19.1	104.4 ± 8.2
	High	92.5 ± 4.5	92.2 ± 3.3	98.1 ± 11.6	101.3 ± 1.4
Baicalin	Low	103.3 ± 5.3	89.4 ± 7.2	104.4 ± 7.5	103.5 ± 3.3
	Medium	105.2 ± 5.7	98.1 ± 4.7	92.8 ± 4.4	103.6 ± 7.8
	High	94.2 ± 5.2	100.8 ± 3.3	90.4 ± 3.3	101.8 ± 1.2

[a]QC: Low, medium, and high were 10, 156, 2500 nM in the bile and 1.56, 25, and 2000 nM in the blood, respectively.

Table 5

The accuracy and intra-day, inter-day precision of wogonosides, apigenin-7-O-glucuronide, and baicalin in the bile and blood matrix.

Analytes	QC[a]	Intra-day				Inter-day			
		Accuracy (%)		Precision (RSD %)		Accuracy (%)		Precision (RSD %)	
		Bile	Blood	Bile	Blood	Bile	Blood	Bile	Blood
Wogonoside	Low	108.4	99.9	2.0	11.2	105.9	94.4	4.4	6.1
	Medium	102.5	99.1	4.3	5.6	104.1	96.3	2.7	7.7
	High	99	96.7	8.3	3.1	99.7	94.1	4.0	4.4
Apigenin-7-O-glucuronide	Low	107.5	111.1	1.7	2.0	111.4	94.5	3.2	5.2
	Medium	113.5	98.7	6.9	12.0	108.0	96.4	4.9	11.0
	High	88.1	94.8	4.7	9.2	95.2	93.0	3.0	10.6
Baicalin	Low	105.9	99.5	4.7	9.9	104.2	92.9	3.2	7.7
	Medium	98.0	94.2	4.8	6.7	102.0	88.5	3.7	10.6
	High	95.3	97.1	7.9	6.9	94.0	94.4	4.8	8.3

[a]Low, medium, and high were 10.0, 156.0, 2500.0 nM in the bile and 1.56, 25.0, and 2000.0 nM in the blood, respectively.