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Simultaneous determination of nine flavonoids in *Polygonum hydropiper* L. samples using nanomagnetic powder three-phase hollow fibre-based liquid-phase microextraction combined with ultrahigh performance liquid chromatography–mass spectrometry

Xian Yang [a], Bo-Chu Wang [a,*], Xue Zhang [b], Shui-Ping Yang [c], Wei Li [a], Qian Tang [a], Gurinder K. Singh [a]

[a] College of Bioengineering, Chongqing University, Chongqing 400044, PR China
[b] National Centre of Biomedical Analysis, Chongqing Academy of Chinese Materia Medica, Chongqing 400065, PR China
[c] Department of Analytical Chemistry, Southwest University, Chongqing 400716, PR China

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ABSTRACT

A simple, inexpensive, and efficient nanomagnetic powder three-phase hollow fibre-based liquid-phase microextraction (HF-LPME) technique combined with ultrahigh performance liquid chromatography–mass spectrometry (UPLC–MS) was developed for the simultaneous analysis of nine flavonoids in *Polygonum hydropiper* L. samples. The final, optimised extraction conditions were as follows: an organic solvent of ethyl acetate, a donor phase of aqueous KH_2PO_4 at pH 3.0, an acceptor phase of aqueous $NaHCO_3$ at pH 8.5, a stirring rate of 1000 rpm, and an extraction time of 50 min. Under these conditions, analyte calibration curves were all linear, with correlation coefficients ≥0.9994. The relative standard deviation for all analytes in intra-day (0.8–2.2%) and inter-day (1.7–3.5%) precision tests was well within the acceptable ranges, as were the limits of quantitation (LOQ < 0.054 μg/L) and detection (LOD < 0.170 μg/L). Recoveries for all standard compounds were between 95.17% and 99.82%, with a RSD of no more than 2.3%. Correlative analyses demonstrated that the physicochemical parameters of the compounds themselves also influenced the extraction efficiency. This technology proved to be rapid, sensitive, and reliable for the quality control of *P. hydropiper* L. samples.

1. Introduction

Polygonum hydropiper L. (Polygonaceae), commonly known as "Laliao", is distributed worldwide in temperate climates and widely found in China [1]. This plant is often used in traditional Chinese medicine (TCM). It contains flavonoid compounds that show promising pharmacological activities (e.g., powerful antioxidant activity that can protect the human body from free radicals) [2–4]. The concentrations of flavonoids in *P. hydropiper* L. are quite low, and it is, therefore, important to establish a simple, sensitive, and effective method to monitor them.

The sample preparation step in an analytical method typically involves an extraction that results in the isolation and enrichment of target analytes from a sample mixture. Non-traditional extraction technologies have been developed to reduce solvent use, increase method automation, and reduce the required sample size [5]. Liquid-liquid extraction (LLE) was developed as a fast, simple, sensitive, and solvent-free sampling and analysis technique [6] and has been widely applied to the sampling and analysis of environmental, biological, and pharmaceutical samples [7–10]. Recently, solid-phase microextraction (SPME), a solvent microextraction procedure, was developed as an alternative sample preparation and enrichment technique for chromatography and electrophoresis [11–13]. To date, the hollow fibre-based liquid-phase microextraction (HF-LPME) approaches that have been developed include two-phase and three-phase HF-LPME. In two-phase HF-LPME, the analytes are extracted by passive diffusion from the sample into a hydrophobic organic solvent, supported by the fibre. In three-phase HF-LPME, the analytes are first extracted through an aqueous solution immobilised in the pores of the fibre, then further extracted into a new aqueous phase in the lumen of the fibre. Several reviews that focus on the basic extraction principles, technical setup, recovery, enrichment, extraction speed, selectivity, applications, and future trends in hollow fibre-based LPME have been reported [14–17].

Compared with LLE and SPME, three-phase HF-LPME gives comparable sensitivity, better enrichment of analytes, and a significant reduction in solvent consumption [10]. Because a small volume of extraction solvent is used, the resulting extracted samples do not require further concentration prior to analysis, and total

* Corresponding author. Tel.: +86 23 65112840; fax: +86 23 65112840.
E-mail address: yzyc09@163.com (B.-C. Wang).

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analysis time is considerably decreased in comparison with traditional SPME procedure.

Additional advantages of HF-LPME make this technique attractive for use in the analysis of many different sample types. Because HF-LPME can tolerate a wide pH range, it can be used in applications that would not be suitable for LLE or SPME. Cross-contamination of samples can be avoided because the fibres are inexpensive enough to dispose of after each use. HF-LPME may also be more economical in situations where a large number of samples need to be analysed. Overall, three-phase HF-LPME has proven to be very useful for both the extraction of metabolites from biological materials and simultaneous cleanup of the extracts [18].

In this study, three-phase HF-LPME was used with ultrahigh performance liquid chromatography–mass spectrometry (UPLC–MS) for the determination of nine flavonoid compounds in *P. hydropiper* L. samples. All parameters were optimised to give a rapid, simple, and sensitive determination of the target analytes. Our results indicate that this method may be useful in the analysis of other TCM samples.

2. Materials and methods

2.1. Chemicals and materials

The nine flavonoid compounds were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their structures are shown in Fig. 1. *P. hydropiper* L. samples were collected from different regions throughout China. Professor Longyun Li (Chongqing Academy of Chinese Materia Medica, China) identified all of the raw medicinal herbs. Chromatography-grade methanol and acetonitrile used for UPLC–MS analysis were purchased from Alltech Scientific (Beijing, China). HPLC-grade water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). n-Octanol, chloroform, ethyl acetate, formic acid, acetone, KH_2PO_4, and $NaHCO_3$ were all AR-grade and purchased from the First Chemical Company of Nanjing (Jiangsu, China).

A 25-μL HPLC microsyringe (Tianjin, China) was rinsed at least five times with the extraction solvent before use. The polyvinylidene fluoride hollow fibre (0.3 mm wall thickness, 0.2 μm pore size, 0.5 mm inner diameter) was purchased from Membrane GmbH (Wuppertal, Germany).

2.2. Chromatography

A Waters UPLC–MS system (Waters, USA) equipped with a dual pump, an autosampler, and a Waters Acquity^{TM} BEH C_{18} analytical column (50 mm × 2.1 mm × 1.7 μm) was used in this study. The aqueous mobile phase was initially composed of 19% methanol (component A) and 0.1% formic acid (component B). Over the first 2 min of the solvent gradient program, the concentration of component A was increased linearly to 40%, where it was held for 9 min. From 12 to 15 min, the concentration of A was linearly decreased from 40% down to 19%, where it was held for 5 min. The mobile phase flow rate was 0.2 mL/min, and the injection volume was 4 μL.

2.3. Mass spectrometry

The electrospray ionisation (ESI) source was operated at a source voltage of 3.5 kV and with a tube compensation lens voltage of 30 V. Nitrogen was used for both the sheath gas (precolumn pressure: 6 Mpa) and the auxiliary gas (precolumn pressure: 0.55 Mpa, flow rate: 10 L/min). The capillary was heated to 350 °C and maintained within a voltage range of 15–50 V. Using the full-scan, positive-ion mode, we monitored ions in the 100–1000 m/z range.

2.4. Sample preparation

Flavonoids were extracted from the plant material in a two-step procedure. First, 0.10 g of dried and powdered *P. hydropiper* L. was transferred to calibrated, amber flasks and extracted with 100 mL of 80% methanol in an ultrasonic bath for 30 min. Additional 80% methanol was added after sonication to compensate for any lost volume, and the resulting solution was filtered through a 0.45-μm

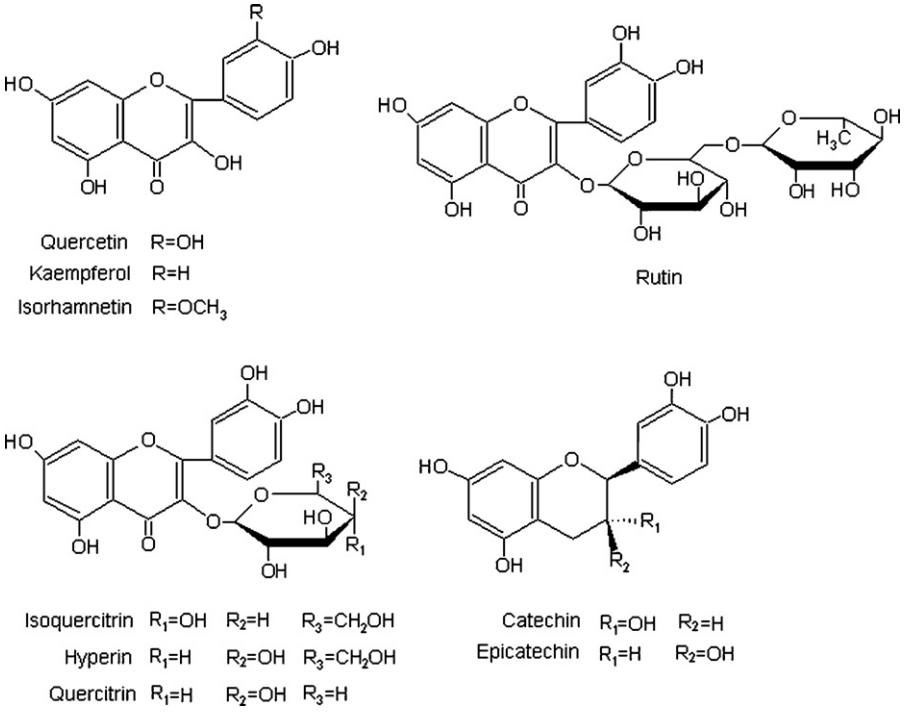

Fig. 1. Chemical structures of nine investigated compounds in *Polygonum hydropiper* L.

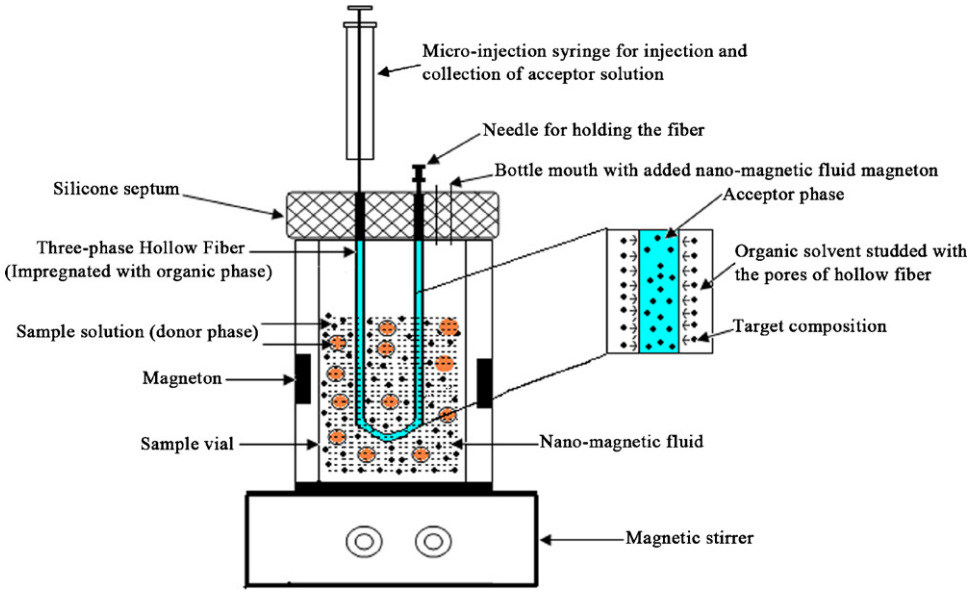

Fig. 2. Basic equipments used for three-phase HF-LPME.

membrane filter. Then, 10.0 mL of the filtered solution was then dried under high-purity nitrogen (Chongqing, China). The residue was re-dissolved in 50 mL of 0.05 mol/L KH_2PO_4 solution (pH 8.5) and further purified with three-phase HF-LPME (Fig. 2). Polyvinylidene fluoride hollow fibres were cut into 7-cm pieces, washed with acetone in an ultrasonic bath, and dried. The fibres were soaked in the membrane phase (ethyl acetate) for 10 s to impregnate the pores of the support. The outside of the fibres was then rinsed with water by placing them into an ultrasonic bath for 30 s to remove any excess organic solvent. The lumen of the prepared fibre piece was filled with 25 μL of the acceptor phase (10 mmol/L $NaHCO_3$ solution; pH 8.5) using a HPLC syringe. Both open ends of the fibre were then closed using the 25-μL HPLC microsyringe and needle. During extraction in a 50-mL sample vial, the membrane portion containing the acceptor phase was immersed in 50 mL of the original extracting solution (pH 3.0). The extracting solution was stirred at 1000 rpm for 50 min with 0.3 g of nanomagnetic powder (200–400 nm diameter, China). After extraction, the fibre was removed, and one of the ends was cut. The acceptor phase was then extracted with the HPLC syringe and injected into the HPLC system.

2.5. Calculation of analysis enrichments

The analyte enrichment factor (EF) is calculated by the following formula:

$$EF = C_{s,\,final}/C_{o,\,initial} \quad (1)$$

where $C_{s,\,final}$ is the final analyte concentration in the acceptor phase, and $C_{o,\,initial}$ is the initial analyte concentration within the sample.

3. Results and discussion

3.1. Optimisation of UPLC–MS conditions

We aimed to both shorten overall run-time and achieve higher separation efficiency and peak resolution of the target compounds by optimising UPLC separation conditions. The use of 1.7-μm porous particles packed into a short (50 mm × 2.1 mm) C_{18} column at a flow rate of 0.2 mL/min yielded an analysis time of less than 20 min and an improved resolution of adjacent peaks. The analysis of a similar sample by conventional HPLC, using a 150 mm (or 250 mm) × 4.6 mm × 5 μm C_{18} column, took more than 30 min [4,19].

We examined the total ion current (TIC) of the nine-flavonoid standard solutions by collecting their MS spectra in full-scan mode. Previous studies have shown that the positive-ion mode produces prominent $[M+H]^+$ ions in the first order mass spectra of the nine flavonoids with higher sensitivity than negative ionisation [20]. Therefore, $[M+H]^+$ ions were selected as the quantitation ions in this study. Source voltage also proved to be an important factor in the quality of MS spectra obtained. Out of 3.0, 3.5, and 4.0 kV used in the positive MS scan mode, the source voltage of 3.5 kV gave a higher signal-to-noise ratio (S/N) for each of the nine compounds.

Table 1
Summary of retention times, quantitation ion and linear equation analysed with the UPLC–MS system.

Analytes	t_R[a] (min)	Quantitation ion $[M+H]^+$ ion (m/z)	Linear range (μg/mL)	Calibration curve	R^2(n = 6)[b]
Rutin	2.491	611.5	0.0237–1.185	$Y = 3.542 \times 10^5 X - 1153$	0.9999
Hyperin	4.022	465.4	0.0131–1.310	$Y = 28.642X + 2.531$	0.9999
Isoquercitrin	5.481	465.3	0.0205–1.025	$Y = 2.684 \times 10^7 X - 2903.9$	0.9999
Quercitrin	7.013	449.4	0.0301–1.505	$Y = 2.191 \times 10^7 X + 139.6$	0.9999
Catechin	8.381	309.3	0.0102–0.510	$Y = 4.192 \times 10^5 X + 6509.7$	0.9999
Epicatechin	9.272	309.3	0.00160–0.160	$Y = 4.613X + 496.5$	0.9999
Quercetin	9.936	339.5	0.00822–1.644	$Y = 2.689 \times 10^{-7} X - 2.898 \times 10^{-4}$	0.9999
Kaempferol	12.462	287.2	0.0405–4.050	$Y = 2.863 \times 10^{-7} X - 2.267 \times 10^{-4}$	0.9999
Isorhamnetin	15.745	317.3	0.0371–1.855	$Y = 3.957 \times 10^6 X + 6.241 \times 10^3$	0.9994

[a] Retention time.
[b] Correlation coefficient.

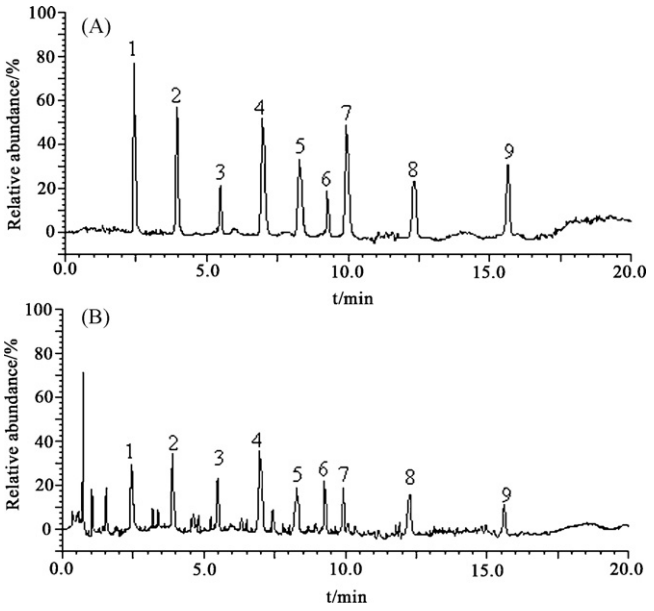

Fig. 3. MS-TIC ([M+H]$^+$ ion mode) chromatograms of the standard solutions (A) and extracts in *Polygonum hydropiper* L. (B). Rutin (1), hyperin (2), isoquercitrin (3), quercitrin (4), catechin (5), epicatechin (6), quercetin (7), kaempferol (8) and isorhamnetin (9).

Finally, setting the capillary voltage to 15–50 V further increased sensitivity and yielded a complete fragmentation pattern for all nine flavonoids. The retention time (t_R) and [M+H]$^+$ ion parameters are shown in Table 1.

Two mobile phase solvent systems, abbreviated as SI and SII, were evaluated to optimise the simultaneous separation of the nine flavonoids. The SI used a methanol:water (50:50, v/v) solution in an isocratic elution program. The SII used an aqueous methanol solution with 0.1% formic acid in the gradient elution program described in Section 2.2. The SII generated more [M+H]$^+$ molecular ions and was therefore used in the final analyses. MS-TIC of the standards and one representative sample are shown in Fig. 3A and B, respectively.

3.2. Method validation

Calibration curve linearity was examined using standard solutions. A mixed stock solution of rutin (59.25 μg/mL), hyperin (65.50 μg/mL), isoquercitrin (51.25 μg/mL), quercitrin (75.25 μg/mL), catechin (25.50 μg/mL), epicatechin (8.00 μg/mL), quercetin (82.20 μg/mL), kaempferol (202.50 μg/mL), isorhamnetin (92.75 μg/mL) was prepared in 80% methanol. From this stock solution, 0.01 (a), 0.02 (b), 0.04 (c), 0.08 (d), 0.20 (e), 0.40 (f), 1.00 (g) and 2.00 (h) mL were transferred to 100-mL volumetric flasks and adjusted to 100 mL with 80% methanol for the standard curve dilutions. Calibration curve of quercetin contained eight different concentrations (a–h). Calibration curves of hyperin, epicatechin and kaempferol contained seven concentrations (b–h). Calibration curves of rutin, isoquercitrin, quercitrin, catechin and isorhamnetin contained six concentrations (c–h). All measurements were made in duplicate. The linear regression equations determined by plotting peak area (Y) versus concentration (X) for each analyte are given in Table 1. All calibration curves showed good linearity ($R^2 \geq 0.9994$) within the concentration ranges tested.

The precision for each compound was evaluated for intra- ($n = 3$) and inter-day ($n = 6$) repeatability. UPLC–MS RSDs (relative standard deviations) for intra-day trials were between 0.8% and 2.2%. Inter-day repetition RSDs for each flavonoid at one of its concentration levels were in the range of 1.7–3.5%. These data are listed in Table 2 and demonstrate that the method had an acceptable degree of precision.

The limits of detection (LOD) and quantification (LOQ) were defined as the concentrations of a compound with signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. Nine flavonoids were determined by serial dilution of a standard solution using the described UPLC–MS conditions. The LODs and the LOQs for the analytes were found to be less than 0.054 μg/L (LOD) and 0.170 μg/L (LOQ) for all nine analytes under the described UPLC–MS conditions (Table 2).

To test the recoveries of the methods, six portions of *P. hydropiper* L. were spiked with the mixed standard solution. The samples were processed as described in Section 2.4, and the results are summarised in Table 2. All recoveries were between 95.17% and 99.82%, with RSDs of 2.3% or less, which is well within acceptable limits (see Table 2).

3.3. Optimisation and evaluation of experimental conditions for three-phase HF-LPME

The nine analytes can be grouped according to their chemical structures. Rutin, hyperin, isoquercitrin, and quercitrin are all flavonoid glycosides. Catechin and epicatechin are flavanols, while quercetin, kaempferol, and isorhamnetin are flavonols [4,21,22]. We chose rutin, catechin, and quercetin as the most representative compounds from each group and used them to optimise the experimental conditions for three-phase HF-LPME.

3.3.1. Organic solvent selection

Selection of a suitable organic solvent for three-phase HF-LPME is important for efficient analyte pre-concentration. An appropriate solvent choice should be easily immobilised in the hollow fibre pores, have low volatility to prevent solvent loss during extraction, and be immiscible with water to serve as an effective barrier

Table 2
Recovery, precision, LOD and LOQ of nine analytes.

Analytes	Recoveries					Intra-day precisions (mg/g)		Inter-day precisions (mg/g)		LOD (μg/L)	LOQ (μg/L)
	Initial (%, μg)	Added (%, μg)	Detected (%, μg)	Average recovery (%)	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)		
Rutin	319.10	309.90	614.25	95.24	1.3	0.633 ± 0.051	1.6	0.611 ± 0.014	2.9	0.030	0.090
Hyperin	186.40	189.50	366.75	95.17	1.6	0.371 ± 0.037	1.9	0.368 ± 0.013	2.6	0.016	0.049
Isoquercitrin	40.30	39.90	79.06	97.14	2.6	0.0790 ± 0.014	1.7	0.0792 ± 0.030	2.8	0.054	0.170
Quercitrin	474.20	478.50	938.89	97.11	1.1	0.945 ± 0.042	2.2	0.911 ± 0.031	1.7	0.002	0.006
Catechin	46.50	46.20	92.01	98.51	1.3	0.0923 ± 0.018	0.8	0.0907 ± 0.025	3.1	0.008	0.026
Epicatechin	42.60	42.20	84.22	98.63	1.8	0.0842 ± 0.021	1.9	0.0846 ± 0.011	2.4	0.010	0.031
Quercetin	191.20	193.00	383.86	99.82	1.4	0.377 ± 0.016	2.1	0.370 ± 0.009	2.6	0.006	0.019
Kaempferol	141.30	145.20	284.68	98.75	1.9	0.281 ± 0.013	1.2	0.277 ± 0.001	3.5	0.003	0.009
Isorhamnetin	14.20	15.00	28.85	97.67	2.3	0.0293 ± 0.002	2.0	0.0288 ± 0.006	1.8	0.020	0.065

Table 3
The quantities of nine analytes from the six batches of *Polygonum hydropiper* L. samples.

Analytes	Content (%, mg/g; $n = 3$)					
	1	2	3	4	5	6
Rutin	0.633 ± 0.020	0.582 ± 0.010	0.691 ± 0.015	0.704 ± 0.007	0.866 ± 0.000	0.935 ± 0.017
Hyperin	0.374 ± 0.011	0.548 ± 0.024	0.467 ± 0.029	0.631 ± 0.026	0.612 ± 0.009	0.494 ± 0.012
Isoquercitrin	0.0796 ± 0.014	0.0924 ± 0.003	0.308 ± 0.014	0.254 ± 0.030	0.277 ± 0.017	0.381 ± 0.026
Quercitrin	0.945 ± 0.032	0.911 ± 0.018	0.804 ± 0.019	0.548 ± 0.032	0.671 ± 0.011	0.593 ± 0.006
Catechin	0.0922 ± 0.015	0.0911 ± 0.017	0.0804 ± 0.021	0.0948 ± 0.020	0.0671 ± 0.023	0.0591 ± 0.019
Epicatechin	0.0846 ± 0.020	0.0849 ± 0.029	0.0720 ± 0.020	0.0816 ± 0.020	0.0529 ± 0.026	0.0601 ± 0.021
Quercetin	0.374 ± 0.022	0.653 ± 0.031	0.275 ± 0.033	0.401 ± 0.032	0.455 ± 0.010	0.397 ± 0.016
Kaempferol	0.284 ± 0.001	0.527 ± 0.003	0.365 ± 0.017	0.281 ± 0.026	0.503 ± 0.007	0.427 ± 0.013
Isorhamnetin	0.0291 ± 0.021	0.0342 ± 0.002	0.0326 ± 0.008	0.0405 ± 0.012	0.0389 ± 0.003	0.0350 ± 0.006

between the two aqueous donor and acceptor phases. The solvent should also be used to promote analyte diffusion from the donor phase into the acceptor phase through the pores of the hollow fibre. Based on these considerations, n-octanol, chloroform, and ethyl acetate were evaluated as the organic solvents for three-phase HF-LPME. The results show that the EFs of rutin, catechin and quercetin, respectively, with ethyl acetate were 53%, 15%, 25% greater than those with n-octanol, which were 25%, 6%, and 20% more efficient than that with chloroform. Therefore, ethyl acetate was selected as the organic extraction solvent for the remaining experiments.

3.3.2. Selection of extraction time, stirring speed, and the quantity of nanomagnetic powder

Rutin, catechin, and quercetin were extracted for different amounts of time (10–60 min) to optimise the extraction efficiency. The purity was checked by UPLC–MS every 10 min. The EFs were calculated according to Section 2.5. The results showed that their EFs increased slowly during the first 20 min, then rapidly increased between 20 and 40 min, and slowly increased again until 50 min. After 50 min, the EFs decreased with increasing extraction time. The EFs of rutin, catechin, and quercetin reached their highest values (32, 56, and 81, respectively) at 50 min, which was the length of extraction time used in subsequent experiments.

To search for the optimal stirring speed, EFs were calculated from 500 to 1100 rpm at 100-rpm intervals. The enrichment enhanced dramatically as speed was increased from 500 to 1000 rpm, then remained constant. The speeds that approached 1100 rpm produced excessive air bubbles. The selected speed of 1000 rpm yielded EFs of 33 (rutin), 57 (catechin), and 80 (quercetin). Therefore, 1000 rpm was selected for subsequent studies.

To select optimal amount of nanomagnetic powder on efficiency, different amounts (0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 g) of nanomagnetic powder was used. The EFs of rutin (31), catechin (55), and quercetin (84) increased with increasing powder weight and reached these maximum values at 0.30 g. Any excess powder resulted in a loss of solvent, and thus, 0.30 g was chosen as a suitable quantity.

3.3.3. Selection of pH

The nine flavonoids in this study are acidic because they contain 5, 7, and 4′ phenolic hydroxyl groups that interface with the neighbouring benzene ring through ρ–π interactions (i.e., conjugation effect). Therefore, changes in pH could change their existing forms (neutral molecular forms or ion forms). In the donor phase, the flavonoids should be in their uncharged form, so that they will be retained in the organic membrane phase when exposed to it. Appropriate amounts of weak acid (0.05 mol/L KH_2PO_4) were added to the donor phase to yield pH values of 2.0, 2.5, 3.0, 3.5, and 4.0. The EFs of rutin (34), catechin (56), and quercetin (80) were highest at pH 3.0. To then retain the flavonoids and prevent diffusion back to the organic solvent, the compounds should be in their charged state once in the acceptor phase. Appropriate amounts of weak base (10 mmol/L $NaHCO_3$) were added to achieve the desired pH levels, ranging from 7.0 to 9.5. Further increases in basicity may have caused degradation of the membrane phase. The $NaHCO_3$ solution with a pH of 8.5 was selected as the best acceptor phase (EFs: rutin, 31; catechin, 54; quercetin, 79).

3.4. Application of optimised method

To validate the method, six batches of *P. hydropiper* L. were extracted using the optimised parameters described in Section 2.4. Table 3 shows that all of the nine flavonoids were reliably identified and quantified in the different batches. Out of the nine compounds, the average content of quercitrin was the highest, followed by rutin, with isorhamnetin as the lowest. The average flavonoid content in the *P. hydropiper* L. samples fluctuated according to the source of the raw materials, treatment of which can vary due to several factors (e.g., cultivation or storage) [23].

3.5. Relationship between EF and other parameters

In addition to the optimised experimental conditions for three-phase HF-LPME, correlative analysis revealed that physicochemical parameters, such as the distribution coefficient (K), number of hydroxyl groups (N), and molecular weight (M) of the flavonoids

Table 4
The result of some parameters of nine analytes in *Polygonum hydropiper* L. samples.

Analytes	Enrichment factor (EF)	Distribution coefficient[a] (K)	The number of hydroxyl (N)	Molecular weight (M)
Rutin	36	1.28	4	610.52
Hyperin	38	1.34	4	464.37
Isoquercitrin	49	1.81	4	464.38
Quercitrin	44	2.03	7	448.38
Catechin	59	2.26	5	308.28
Epicatechin	66	2.37	5	308.28
Quercetin	83	2.98	5	338.27
Kaempferol	71	2.56	4	286.23
Isorhamnetin	58	2.18	4	316.27

[a] *Note*: ethyl acetate/water distribution coefficient was determined by shake-flask method.

themselves, also influenced analyte extraction efficiency. EF and physicochemical parameters of the analytes are listed in Table 4.

Regression analyses show the influence of *K*, *N*, and *M* on EF. There was a significantly high positive correlation between *K* and EF ($r = 0.964$, $p < 0.01$), with a linear regression relationship of $y = 27.56x - 1.606$ ($R^2 = 0.9301$). *M* was negatively correlated with EF ($r = -0.810$, $p < 0.01$) and had a linear regression relationship of $y = -0.12x + 102.06$ ($R^2 = 0.6562$). However, there was no significant correlation between EF and *N*. Thus, the EFs of flavonoids in three-phase HF-LPME not only depend on the choice of extraction conditions but also on the physicochemical properties of the compounds themselves. Among these properties, the distribution coefficient, *K*, has the most influence on EF.

4. Conclusions

We have developed and validated a rapid, sensitive, and reliable method for the quantitative determination of nine flavonoid compounds in *P. hydropiper* L. using HF-LPME combined with UPLC–MS. Our results indicate that three-phase technology is a good alternative extraction technique and offers many analytical advantages (e.g., extracting and pre-concentrating analytes of different polarities) over traditional methods. With low detection limits, good reproducibility, and a wide range of linearity, the method can reliably be used in quality control of *P. hydropiper* L. samples.

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