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Effervescent salt and crown ether-assisted matrix solid-phase dispersion extraction of coumarins from *Cortex fraxini*

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ABSTRACT

A simple, rapid and environmentally friendly method named effervescence-assisted matrix solid-phase dispersion (EA-MSPD) was investigated for the extraction of four coumarins (esculin, esculetin, fraxin and fraxetin) from *Crotex fraxini* (*C. fraxini*). In this study, an effervescent tablet containing carbon dioxide sources (100 mg sodium bicarbonate and 200 mg sodium dihydrogenphosphate) and an adsorbent (25 mg benzo-15-crown-5) was prepared. The effervescent tablet was dissolved into an aqueous solution, and as a consequence of the effervescence reaction, carbon dioxide was generated, thus making the sample better a dispersion of the extraction solvent (100 mM sodium dodecyl sulfate). The variables that influenced the extraction efficiency included effervescent salts, extraction solvents and crown ether. These values were represented using ultra- high-performance liquid chromatography (UHPLC). Good linearity was exhibited by coefficients of determination all equal to 1, limits of detection ranging from 1.62 to 3.83 ng·mL^{-1} and limits of quantification ranging from 5.39 to 12.76 ng·mL^{-1}. The recoveries ranged from 91.37 to 100.29% with relative standard deviations of 0.57–4.58% under optimized conditions. In this work, the proposed EA-MSPD method was successfully applied to extract and identify four coumarins in *C. fraxini*. Compared with previously reported methods, the method examined herein was faster, greener and more sensitive.

1. Introduction

Crown ethers (CEs), which are notable for their unique property of forming stable complexes with alkali metal ions $[MCE]^{+}$ or $[MCE]^{2+}$, were first developed by Pedersen in 1967 (Pedersen, 1967). CEs can strongly solvate cations because of their ability to selectively interact with cations in the crown cavity and a nearly planar arrangement of oxygen atoms around the central cation (Kumbhat and Singh, 2018). The stability of the CE and metal ion complex is mainly governed by the host-guest bonding match relationship between the diameter of the metal ion and the size of the interior of CE (Robak et al., 2006). The oxygen atom in the CE planar structure coordinates with the cation interior of the ring, while the exterior of the ring is hydrophobic (Zhou et al., 2007). Therefore, it was typical to utilize the hydrophobicity of the CE to select a suitable cavity size for selective and efficient extraction of a particular metal ion. However, compounds that have a discrete crown structure are dissolved in most organic reagents and thus exhibit chemical instability (Chen et al., 2016). The proposed method shows CEs have been anchored on the polymer backbone and are stable in reagents (Ahmadi et al., 2016). In recent studies, CEs have been applied in novel chromatographic extraction methods (Li et al., 2018), food and environmental determinations (Blanchet-Chouinard and Larivière, 2018; González-Calabuig et al., 2016), functionalized electrochemical sensors (Zhang et al., 2017) and chiral separations (Hyun, 2016). In this experiment, a new method was developed to extract coumarin compounds from the natural medicinal plant *Crotex fraxini* (*C. fraxini*) by using the cavity and hydrophobic properties of the CE as an adsorbent.

Matrix solid-phase dispersion (MSPD) was a promising technique for sample pretreatment process (Barker et al., 1989). The extraction process consisted of three main steps: blending sample and dispersing material, transferring material to the syringe barrel and compressing the material, and elution (Capriotti et al., 2015). Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are widely used methods of sample preparation for separating target analytes. However, all of these extraction methods share common drawbacks such as needing sample homogenization and tissue debris removal prior to column application, difficulty separating impurities and requiring subsequent clean-up.

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MSPD was a sample preparation technique for the simultaneous accomplishment of both extraction and clean-up steps, which overcomes the complications of LLE and SPE for solid samples and consequently simplifies the pretreatment process and shortens extraction time (Yin et al., 2012). Its main advantages over other conventional methods of sample preparation include reduction of organic solvent consumption, short extraction times, improvement of extraction efficiency and clean-up of the sample before chromatographic analysis (Ramos et al., 2008). The method of MSPD assisted extraction has been applied to various fields, including natural product extraction (Peng et al., 2016; Wianowska and Dawidowicz, 2016), drug analysis (León-González and Rosales-Conrado, 2017; Argente-García et al., 2016), food safety (Chen et al., 2017; Tan et al., 2017; Wang et al., 2018a, 2018b) and pesticide residue determination (Lozowicka et al., 2016). However, conventional MSPD was used in conjunction with SPE to achieve the purpose of extraction. Due to the low selectivity of common adsorbents (C_{18}, C_8, silica gel, florisil, etc.), conventional MSPD has shown low extraction efficiency for low level analytes, and the process of filling the columns is complicated and time consuming (Wang et al., 2018a, 2018b). In addition, MSPD uses toxic organic reagents (acetonitrile, hexane, methanol et.al) during sample pretreatment, and still requires a large amount of sample. Therefore, it is necessary to develop a column-free method with a special adsorbent for extracting complex components.

C. fraxini, commonly referred to Qinpi, is recorded in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015) and is derived from the dry bark of Fraxinus rhynchophylla Hance, Fraxinus chinensis Roxb, Fraxinus szaboana Lingelsh and Fraxinus stylosa Lingelsh, a commonly used traditional Chinese herbal medicine. *C. fraxini* as a common traditional Chinese herbal medicine; the main effect is to inhibit inflammation, bacterial dysentery and relieve fever, asthma and cough. It is commonly used for the treatment of gout, arthritis, diarrhea and bacillary dysentery in clinics (Chinese Pharmacopoeia Commission, 2015). *C. fraxini* contains a variety of chemical components, including coumarins, lignans, phenylpropanols, iridoids and phenolic compounds, while coumarins are considered to be the main biologically active ingredients that induce the pharmacological effects of *C. fraxini*. esculin, fraxin, esculetin and fraxetin are the index components of quality control of *C. fraxini* medicinal materials, and the chemical natures are shown in Fig. 1. In this study, the content of esculin, fraxin, esculetin and fraxetin in Qinpi have been determined in Table 2, which are 9.45 mg/g, 8.09 mg/g, 1.36 mg/g and 0.36 mg/g, respectively. Conventional extraction methods including ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) not only use toxic organic solventsbut also require long extraction times (Liu et al., 2015; Zhou et al., 2011). The recoveries of esculin and esculetin were determined by the extraction methods used so far, which were 98.8% (added amount 8.00 mg/g), 95.2% (added amount 5.00 mg/g) (Zhou et al., 2011) and 99.67% (concentration 2.46 mg/mL), 99.75% (concentration 1.03 mg/mL) (Liu et al., 2015), respectively. Therefore, it is particularly important to develop an extraction method in aqueous solution that leads to an efficient, convenient and simultaneously determination of a variety of coumarins.

In this paper, an effervescence-assisted method, which was applied in the matrix solid-phase dispersion, has been presented for the extraction of four coumarins (esculin, esculetin, fraxin and fraxetin) in *C. fraxini*. A series of variables was designed for optimizing conditions, including effervescent salt, extraction solvent and adsorbent. Then, the analytes were analyzed via UHPLC. The proposed method could detect hydrophobic compounds (coumarins) in an aqueous solution without the use of toxic organic chemicals making it therefore more environmentally friendly and diffusely applied.

2. Material and methods

2.1. Chemicals and reagents

Sodiumcarbonate, sodium bicarbonate, 2-hydroxymethy-12-crown-4 and citric acid were supplied by Alfa Aesar China (Tianjin, China). Sodium dihydrogenphosphate was purchased from Sigma–Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). 15-crown-5 and benzo-15-crown-5 were obtained from Energy Chemical Co., Ltd. (Shanghai, China). Ascorbic acid, sodium dodecyl sulfate and18-crown-6 were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), Acros Organics Co., Ltd. (Shanghai, China) and Aladdin Co., Ltd. (Shanghai, China) respectively. Triton X-100 and 1-Dodecyl-3-methylimidazolium Bromide were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Methanol, acetonitrile and isopropanol (HPLC grade) were supplied by Tedia Company Inc. (Fairfield, US). Analytical standards of esculin, fraxin, esculetin, and fraxetin with a purity of more than 98% were obtained from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). Purified water was provided by the Wahaha Group Co., Ltd. (Hangzhou, China) throughout the experiment. Samples of *C. fraxini* was purchased from Anhui, China.

2.2. Chromatographic analysis

The sample analysis was carried out on an Agilent 1290 UHPLC system (Agilent Technologies, Santa Clara, CA), which consisted of a G4220B 1290 Bin Pump VL, a G1314E 1290 VWD, a G1316C 1290 TCC, and a G4226A 1290 Sampler. Chromatographic separation was performed on reverse-phase SB-C_{18} column (1.8 μm particle size, 50 × 4.6 mm i.d.). The column temperature was maintained at 38 °C. The mobile phase was composed of 0.1% formic acid aqueous (A) and methanol (B) with a flow rate of 0.4 ml/min. According to previous reports (Liu et al., 2014), the gradient procedure was optimized as follows: 0–2 min, 25–40% B; 2–3 min, 40–50% B; 3–7 min, 50% B; 7–8 min, 50–75% B; 8–9 min, 75–100% B; 9–10 min, 100-25% B. The injection volume was 1 μL. The detection wavelength was under 334 nm. The standard solutions were prepared by dissolving each of them (500 μg mL^{-1}) in methanol. Working mixed standard solutions were prepared by dilution of the stock standards in methanol before use. Standard calibration curves were performed using four standard

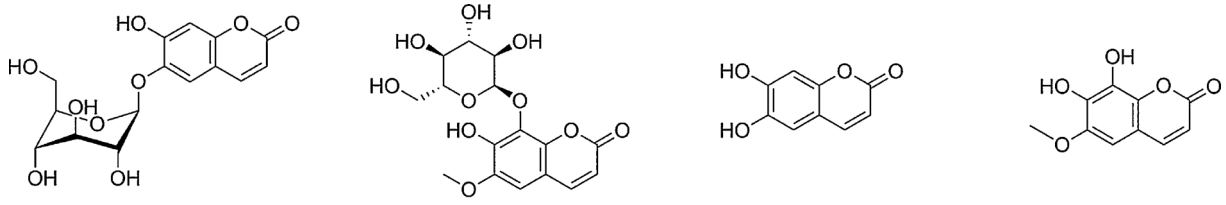

Fig. 1. Chemical properties of compounds.
Esculin: mw: 340.2821, density: 1.679 g/cm^3, solubility in water: moderately soluble; Fraxin: mw: 370.3081, density: 1.634 g/cm^3, solubility in water: none; Esculetin: mw: 216.663, density: 1.207 g/cm^3, solubility in water: slightly soluble; Fraxetin: mw: 208.1675, density: 1.508 g/cm^3, solubility in water: none.

Fig. 2. Description of the general analytical procedure of the effervescence-assisted matrix solid-phase dispersion.

solutions in concentrations ranging from 0.1 to 100 μg mL^{-1} for the sample. The recovery of the samples mixed with the target analytes spiked at two standard concentration levels, 1 and 30 μg·mL^{-1}.

2.3. Sample preparation

The samples of *C. fraxini* were dried in an oven at 60 °C for 12 h, crushed and passed through a 50 mesh sieve plate. Additionally, 200 mg of sodium dihydrogenphosphate and 100 mg of sodium bicarbonate were previously dried at 60 °C for 1 h. Then, 25 mg of CE and 25 mg *C. fraxini* were added, and the mixture was admixed in an agate mortar for 5 min until a homogeneous powder was achieved. A precisely weighed amount of 300 mg for each tablet was condensed in a manual tableting machine at 22 MPa for 2.5 min. Then an effervescent tablet was obtained.

2.4. Extraction procedure

According to previous work (Ye et al., 2015), the extraction procedure was performed, as shown in Fig. 2. At the beginning, the obtained tablets were dissolved effervescently in 2.5 mL purified water with effervescence taking place instantly at the bottom of a wide-mouthed bottle. Then, carbon dioxide was produced from the effervescent tablet to disperse the sample into the aqueous solution homogeneously. When the dissolution process ended, all of sample solution was taken into a 5 mL centrifuge tube by a pipette. The sample solution was centrifuged at 13,000 rpm for 5min. The supernatant was transferred to a 5 mL disposable syringe, and a 0.22 μm filter was used to eliminate other large particle impurities. The sample was transferred into a 2 mL liquid injection bottle for UHPLC analysis.

2.5. Statistical analysis

In this work, the sample solution was extracted three times under optimal conditions, and the standard deviation was calculated according to the following formula:

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - \bar{X})^2} \quad (1)$$

Response surface methodology (RSM) was used to evaluate three factors: concentration of extraction solvent (A), amount of absorbent (B) and grinding time (C). A Box-Behnken Design (BBD) included 17 tests and three repetitions at the central point variables. To normalize factors, the coded values of independent practical variables were fixed at 3 levels (−1, 0, 1). BBD was established as shown in Table 1. The model was illustrated by means of the following quadratic equation:

$$Y = a_0 + a \cdot A + b \cdot B + c \cdot C + d \cdot A^2 + e \cdot B^2 + f \cdot C^2 + g \cdot AB + h \cdot AC + i \cdot BC$$

where Y is the response, A, B and C are the individual factors, and a_0-i are the coefficients of the polynomial equation. Analysis of variance (ANOVA) was performed to evaluate the data of the optimization experiments.

2.6. Method validation

Linear regression was performed using four standard solutions in sample concentrations ranging from 0.1 to 100 μg mL^{-1}. The limits of detection (LOD) and limits of quantification (LOQ) were evaluated as the minimum concentration of coumarins in *C. fraxini* samples on the basis of signal-to-noise ratios (S/N) of three and ten, respectively. Determination of intraday and interday precision were performed by repeated injection of standard solution. Four standard solutions were analyzed at six consecutive times for intraday precision and six consecutive days for interday precision to evaluate the reproducibility of the effervescence-assisted matrix solid-phase dispersion extraction procedure under optimal conditions.

Table 1
Experimental domain of BBD.

Symbol	Independent variable	Coded levels		
		−1	0	1
A	Concentration of extraction solvent (mM)	50	100	150
B	Amount of absorbent (mg)	20	25	30
C	Grinding time (min)	1	3	5

Table 2
ANOVA of response surface model and predicted results for response of four analytes.

Source	Esculin		Fraxin		Esculetin		Fraxetin	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value
Model	143.02	< 0.0001	57.19	< 0.0001	92.45	< 0.0001	42.57	< 0.0001
A	520.41	< 0.0001	132.22	< 0.0001	105.07	< 0.0001	5.19	0.0567
B	47.97	0.0002	8.82	0.0208	198.78	< 0.0001	10.42	0.0145
C	0.048	0.8330	4.11	0.0823	9.33	0.0185	30.42	0.0009
AB	1.44	0.2688	0.19	0.6733	9.41	0.0181	9.26	0.0188
AC	25.09	0.0015	0.12	0.7402	0.90	0.3736	4.97	0.0610
BC	22.44	0.0021	19.39	0.0031	8.63	0.0218	10.23	0.0151
A^2	459.70	< 0.0001	236.42	< 0.0001	306.59	< 0.0001	230.89	< 0.0001
B^2	35.04	0.0006	6.58	0.0373	1.24	0.3027	41.32	0.0004
C^2	163.21	< 0.0001	95.30	< 0.0001	160.76	< 0.0001	17.68	0.0040
Lack of Fit	3.33	0.1376	5.20	0.0726	5.15	0.0736	115.03	0.0002
Adjusted R^2	0.9876		0.9693		0.9809		0.9590	
Std. Dev.	6.70		5.80		1.16		0.81	

3. Results and discussion

3.1. Selection of effervescent salt

The preparation of the effervescent tablet was a key step in the whole experiment. According to the previous research work (Ye et al., 2015), sodium carbonate and sodium bicarbonate were selected as the carbon dioxide sources, and citric acid, ascorbic acid and sodium dihydrogenphosphate were used as the acid components, and their performance was evaluated during the study. The six combinations of two sources of carbon dioxide and three acidic components were dissolved in the solution. It was observed that the effervescence effect of sodium carbonate was far less intense than sodium bicarbonate. Considering the shorter effervescence time decreases the extraction efficiency, the combination of sodium bicarbonate and acid components were further investigated, and the results are shown in Fig. 3a. It can be seen that sodium dihydrogen phosphate exhibited the best extraction efficiency because citric acid and ascorbic acid are hygroscopic and react easily with alkaline salts. A reasonable explanation for this phenomenon is that dissolving effervescent tablets into an aqueous solution can provide a carbon dioxide source to aid the dispersion of the extraction solvent and increase the metastasis of the analytes, which causes an exaltation of extraction efficiency (Wang et al., 2015). In other similar studies, sodium dihydrogen phosphate was selected as a proton donor and the source of carbon dioxide was sodium carbonate (Lasarte-Aragonés et al., 2013). However, the coumarins were unstable in alkaline form and the effervescent combination of the sodium carbonate exhibited weak alkalinity. Due to the effervescence performance and chemical stability, the sodium bicarbonate and sodium dihydrogen phosphate combination was selected as the optimum composition for the following experiment.

3.2. Optimization of the extraction solvent

The type of extraction solvent was a critical parameter that affected the performance of the EA-MSPD method. After selecting the components and amount of the effervescent mixture, three types of extraction solvents were investigated: Triton X-100, Sodium dodecyl sulfate (SDS) and 1-Dodecyl-3-methylimidazolium bromide ($[C_{12}mim][Br]$). The dispersed droplets of the extraction solvents adhered to the generated effervescence causing them to float and gather on the surface of the sample and transfer analytes from solvent to extraction solvent efficiently. The results obtained are shown in Fig. 3b. The extraction efficiency obtained by SDS was slightly higher than that obtained by $[C_{12}mim][Br]$ and markedly higher than obtained by Triton X-100. The main reason for higher extraction efficiency may be that SDS has higher solubility in pure water which positively influenced the transfer of the target compounds from the sample matrix to the extractant at the selected concentration level. Furthermore, the viscosity of the SDS was lower than other tested solvents, which caused rapid dispersion and highly efficient mass transfer (Scholz et al., 2018). Due to the high efficiency of extraction, low viscosity and relatively low toxicity, SDS was selected as the extraction solvent for the next investigation.

3.3. Concentration of the extraction solvent

A reasonable concentration of extraction solvent achieved a high efficiency extraction. SDS could form micelles at critical micelle concentration (CMC). A key property of micelles is to enhance the solubility of hydrophobic compounds in aqueous solutions. It was necessary to find a balance between the concentration and viscosity of the high extraction efficiency because the concentration and viscosity of SDS are positively correlated. To evaluate the effect of concentration, a series of concentrations (50, 100, 150, 200, 250 mM) were studied, and the experimental results are shown in Fig. 3c. It can be seen that the extraction yields increased with concentration from 50 to 100 mM and reached a maximum at 150 mM, after which the peak areas decreased. The extraction efficiencies were nearly equal when the content of extraction solvent was 100 mM and 150 mM, but 150 mM was more viscous than 100 mM. A reasonable explanation is that the shape and size of the SDS were changed at 10 times or higher the CMC, which increased the dissolution of target analytes (Jiang et al., 2014). Considering the solution viscosity of the 150 mM sample, 100 mM was selected as the best concentration of extraction solvent for further studies.

3.4. Type of absorbent

The microporous adsorbent played an important role in assisting dispersion to increase contact between active compounds and the extraction solvent. To increase intermolecular forces between the adsorbent and the target compounds, microporous adsorbent should be carefully selected in the MSPD process. In this study, the CEs acted not only as an adsorbent material but also as carriers for dispersing the sample during the extraction process. Four kinds of microporous adsorbents, including 15-crown-5, 18-crown-6, benzo-15-crown-5 and 2-hydroxymethy-12-crown-4 were investigated in this section. The corresponding results obtained are shown in Fig. 3d. The peak areas obtained with 15-crown-5 and 2-hydroxymethy-12-crown-4 were lower because they were liquid resulting in insufficient grinding. The other two adsorbents provided satisfactory extraction efficiency, and benzo-15-crown-5 demonstrated the highest extraction yields. A possible reason is that the structure of benzo-15-crown-5 led to the interaction between the adsorbent and tested analytes to yield a polar force instead of a nonpolar force, thereby enhancing the adsorption site of the

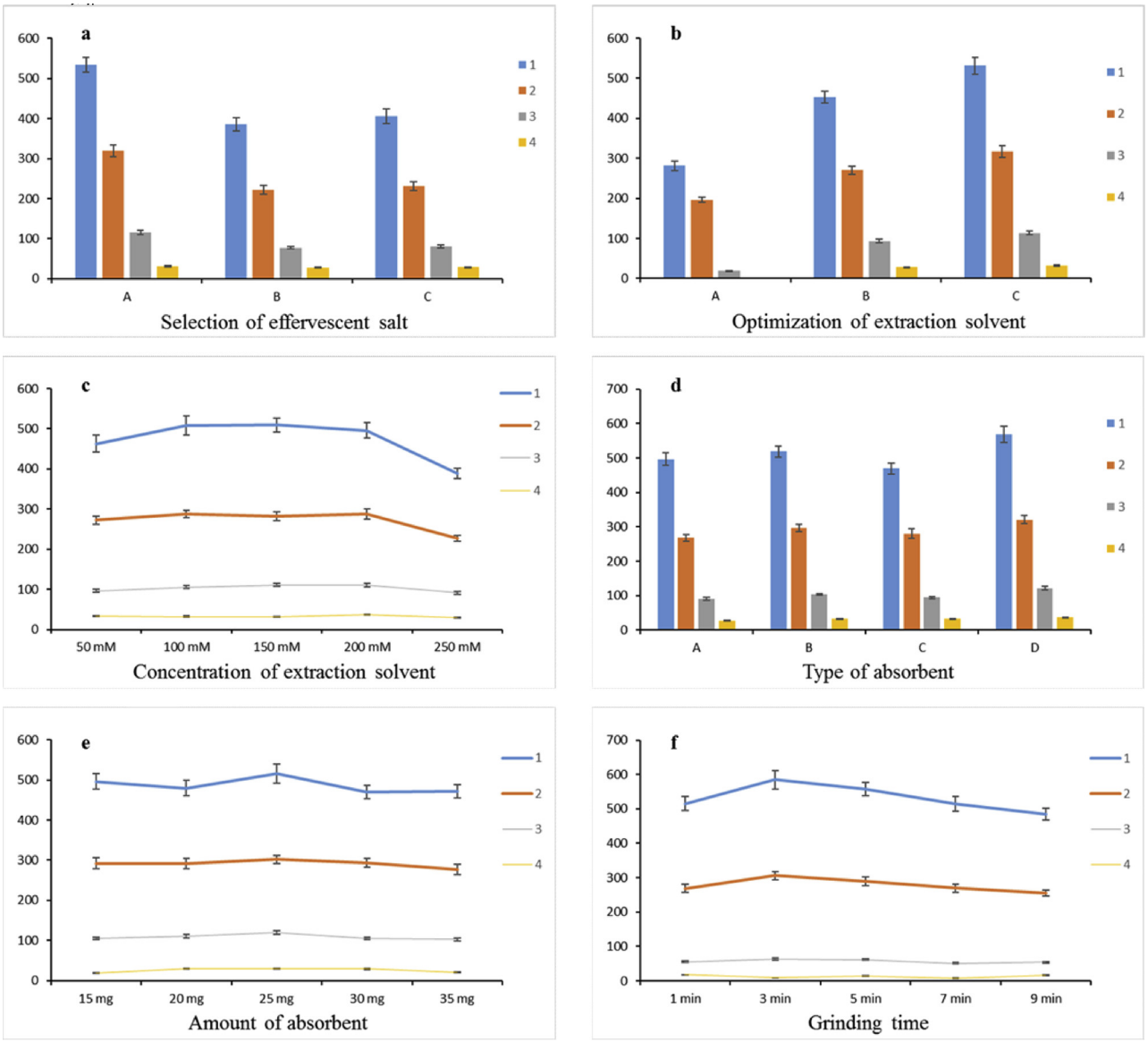

Fig. 3. Optimization of the extraction process.
(a) Selection of effervescent salt: (A) sodium dihydrogenphosphate, (B) citric acid, (C) ascorbic acid. (b) Optimization of the extraction solvent: (A) Triton X-100, (B) 1-Dodecyl-3-methylimidazolium bromide, (C) Sodium dodecyl sulfate. (c) Concentration of extraction solvent. (d) Type of adsorbent: (A) 15-crown-5, (B) 2-hydroxymethy-12-crown-4, (C) 18-crown-6, (D) benzo-15-crown-5. (e) Amount of adsorbent. (f) Grinding time. Analytes: (1) Esculin, (2) Fraxin, (3) Esculetin, (4) Fraxetin.

adsorbent materials to the target compounds (Kurahashi et al., 2008). Therefore, benzo-15-crown-5 was selected as the optimal absorbent material in the following experiments.

3.5. Amount of absorbent

The CEs had a large specific surface area and therefore provided satisfactory absorption capacity during the procedure of microextraction. In the study, an amount of benzo-15-crown-5 ranging from 15 to 35 mg was tested. Fig. 3e indicates that when the amount was less than 25 mg, the extraction efficiency increased as the amount of benzo-15-crown-5 increased, and when the amount was larger than 25 mg, the extraction efficiency slightly decreased. An excessively low amount of adsorbent could be insufficient for the extraction and transfer of analytes from the matrix to solvent. The results of this experiment can be explained by the fact that a larger amount of adsorbent material had a stronger interaction with the analyzed compounds, thereby increasing the extraction yield. Furthermore, it should be emphasized that higher dose of CEs could reduce the cohesion between the tablet component affecting the physical stability of the effervescent tablets. On the one hand, agglomeration occurred resulting in the decline in extraction efficiency when an excessive amount of CE was used in the extraction process. On the other hand, the excessively large amount of microporous adsorbent could lead to long extraction time and, possibly, the partial decomposition of analytes (Wang et al., 2015). To sum up, the extraction efficiency of benzo-15-crown-5 was highest when the amount of the absorbent was 25 mg. Thus, 25 mg benzo-15-crown-5 was used in subsequent experiments.

3.6. Grinding time

Grinding time was an important factor in the selective extraction of C. fraxini in the foaming salt tableting method using MSPD (matrix solid phase dispersion). To investigate the effect of the grinding time on the extraction efficiency, a grinding test was carried out at different time intervals (1.0–9.0 minutes). The experimental results are shown in

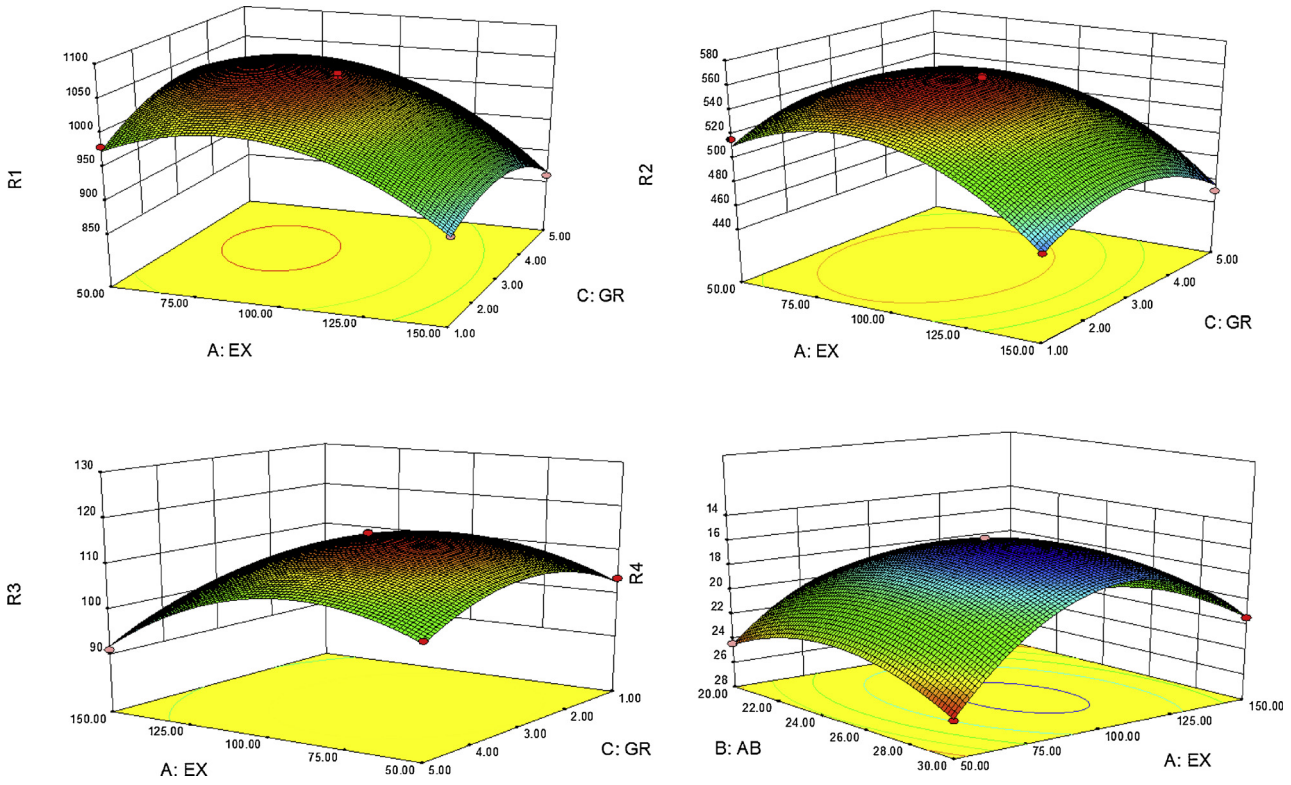

Fig. 4. The three-dimensional response surface plots.
R1: Esculin, R2: Fraxin, R3: Esculetin, R4: Fraxetin. Three single factors: (A) Concentration of extraction solvent, (B) Amount of absorbent (C) Grinding time.

Table 3
Method validation.

Linear Regression Data, Precision, Reproducibility, Limits of detection (LODs) and Limits of quantification (LOQs) of the Investigated Compounds										
Analyte	Calibration curve				Precision (RSD%)				LOD	LOQ
	Calibration levels (n = 9)				Intraday n = 6		Interday n = 4		ng mL^{-1}	ng mL^{-1}
	r^2	Slopes	Intercepts	Linear ranges μg mL^{-1}	Retention time	Peak area	Retention time	Peak area		
Esculin	1.0000	5.45 ± 0.24	0.68 ± 0.05	0.1–100	0.045	0.86	0.112	1.32	1.62	5.39
Fraxin	1.0000	3.73 ± 0.15	0.29 ± 0.02	0.1–100	0.043	0.79	0.068	1.20	2.62	8.73
Esculetin	1.0000	8.80 ± 0.40	−0.01 ± 0.02	0.1–100	0.034	0.64	0.108	0.67	2.77	9.24
Fraxetin	1.0000	8.30 ± 0.38	−0.36 ± 0.04	0.1–100	0.026	0.57	0.069	0.68	3.83	12.76

Table 4
Sample Analysis.

Content, Average Recovery and Reproducibility of Samples.						
Analyte	Content		Added(μg/mL)	Recovery%	Reproducibility (sample	
	Crotex fraxini	Crotex fraxini		Crotex fraxini	extraction) (RSD%) n = 3	
	injection(μg/mL)	(mg/g)			Retention time	Peak area
Esculin	94.50	9.45	0.1	93.42 ± 3.86	0.019	2.26
			5	100.29 ± 4.50		
Fraxin	80.88	8.09	0.1	91.55 ± 4.21	0.016	2.10
			5	96.36 ± 4.62		
Esculetin	13.56	1.36	0.1	92.12 ± 4.51	0.014	4.35
			5	92.77 ± 3.70		
Fraxetin	3.56	0.36	0.1	98.23 ± 3.18	0.013	4.58
			5	91.37 ± 3.92		

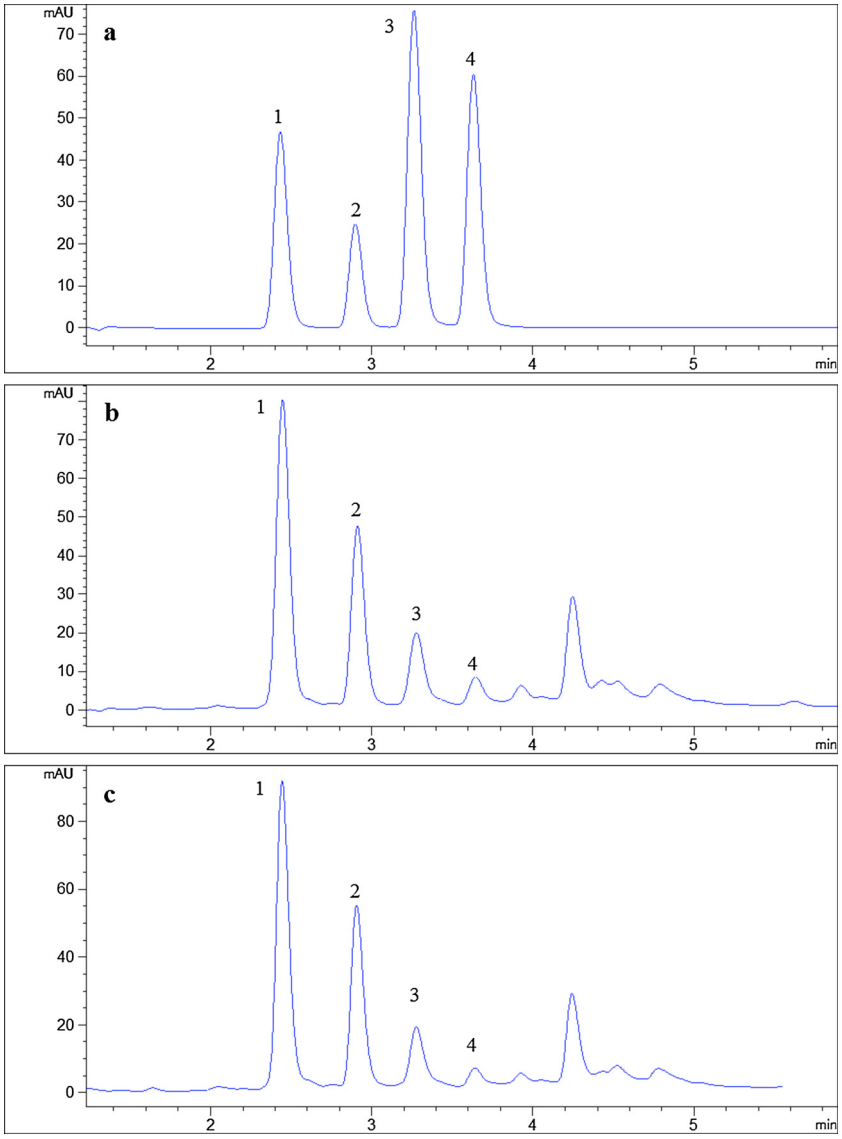

Fig. 5. Chromatogram results.
(a) Standard solution. (b) Sample dissolved in methanol. (c) Sample dissolved in effervescent tablet solution. Target analytes: (1) Esculin, (2) Fraxin, (3) Esculetin and (4) Fraxetin.

Fig. 3f. The extraction efficiency of the four analytes slightly increased when the grinding time increased from 1 min to 3 min. Next, the peak areas of the four target analytes began to decline, but the trend was less obvious when the grinding time was extended to 9 min. However, if the time was too short, the target analytes in the sample mixture risked not extracted entirely, leading to a decrease in the extraction efficiency. Considering the effect of grinding time, prolonged grinding could not affect the extraction efficiency significantly; instead, it will increase the risk of matrix effect from lipids and other interfering compounds. Compared to the previous study, and given the mixing state of the material at a grinding time of 3 min, 3 min of grinding time was considered sufficient for subsequent studies.

3.7. Response surface methodology and model fit

A BBD project designed for EA-MSPD experiments was expressed for statistical analysis and model fitting to predict optimum experimental conditions and evaluate reliability and acceptability of models. Therefore, RSM was used to confirm considerable influencing factors such as concentration of extraction solvent (A, 50–150 mM), amount of absorbent (B, 20.0–30.0 mg) and grinding time (C, 1–5 min). The final models for four target analytes are shown as follows:

Esculin = 1048.72-54.05 * A 16.41 * B-0.52 * C-4.03 * AB-16.79 * AC-15.87 * BC-70.03 * A^2 19.33 * B^2-41.73 * C^2

Fraxin = 555.12-23.57 * A 6.09 * B-4.15 * C 1.27 * AB-1.00 *AC-12.76 * BC-43.44 * A^2 7.24 * B^2-27.58 * C^2

Esculetin = 114.78-4.19 * A 5.77 * B-1.25 * C 1.77 * AB-0.55 * AC-1.70 * BC-9.88 * A^2-0.63 * B^2-7.15 * C^2

Fraxetin = 15.64-0.65 * A-0.92 * B 1.57 * C-1.23 * AB-0.90 * AC 1.29 * BC 5.96 * A^2 2.52 * B^2 1.65 * C^2

Suitability and evaluation of the regression model was also analyzed by means of the analysis of variance (ANOVA) test. P-values were applied to assess the statistical significance in order to understand the mode of mutual interactions between the three variables. P-values less than 0.05 demonstrated that the mathematical models were considered to be significant, while p-values higher than 0.05 were insignificant. As shown in Table 2, the regression coefficients, p-values and F-values are listed.

To show the relationship between the variables studied, a three-dimensional (3D) response surface plot was drafted, and the effects of

Table 5
Comparison of miniaturized MSPE with previously reported methods.

Analytes	Sample	Instrumental technique[a]	Extraction method[b]	Sample amount	Extraction solvent[c]	Solvent volume	Analysis time	Extraction time	LODs	Advantages	Disadvantages	Reference
Aesculin Aesculetin	*C. fraxini*	HPLC	ILSMP-UMSE	1.0 g	LiAc	2 wt%	10 min	20 min	4.4-5.6 μg/ml	High extraction efficiency and good recoveries (98.83-99.49%)	Complicated operation, makes use of toxic reagent (ILs), low sensitivity	Liu et al., 2015
Nevadensi Aesculin Aesculetin	*C. fraxini*	HPLC	MAE	1.0 g	PEG-200	10:1(liquid/solid)	10 min	10 min	0.06-0.09 ug/ml	Uses nontoxic reagents and less extraction time	Waste energy and makes chromatogram impurity	Zhou et al., 2011
Esculin Esculetin	*C. fraxini*	CE	UAE	1.0 g	Methanol	20 mL	9 min	30 min	0.22-0.27 mM	Lower detection potentials (10.80 V) and good precision (RSD 3.7%)	Makes use of organic reagent (methanol), high pretreatment time (> 60 min) and amount of samples (1.0 g)	Chen et al., 2009
Esculetin	*C. fraxini*	HPLC-MS/MS	UAE	0.2 g	80% Methanol	25 mL	15 min	45 min	0.02 ng/mL	High Sensitivity (0.02 ng/mL) and good recoveries (102.0-110.2%)	Makes use of organic reagent (methanol), consumes extraction solvent volume (30 mL), analysis time (45 min)	Yun et al., 2011
Aesculin Fraxin Aesculetin Fraxetin	*C. fraxini*	UHPLC	MSPD	0.025 g	SDS	100 mM	9 min	10 min	1.62 to 3.83 ng/mL	Good recoveries (91.37-100.29%), high sensitivity, less consumption of samples, no use of organic solvents for extraction, short extraction time and analysis time		This method

[a] HPLC high-performance liquid chromatography. CE capillary electrophoresis. UHPLC ultrahigh-performance liquid chromatography. LC–MS/MS liquid chromatography–tandem mass spectrometry.
[b] ILSMP-UMSE ionic liquids microwave pretreatment-ultrasound microwave synergy extraction, UAE ultrasonic-assisted extraction, MAE microwave-assisted extraction, MSPD, matrix solid-phase dispersion.

the responses and interactions of the two variables on the extraction yield of the target analyte were evaluated. Two variables varying in the range of the experiment under investigation were described while the other variables were kept at central level (0 level). Therefore, extraction yields of four coumarins aff ;ected by concentration of extraction solvent (A), amount of absorbent (B) and grinding time (C) are shown in Fig. 4.

As seen from the statistical analysis results, the elements with better eff ;ects on extraction of esculin were A and B, and interactional elements AC and BC ($p < 0.05$) while AB ($p > 0.05$) was not significant. For fraxin and esculetin, the interactional elements with better effects on extraction yield were BC and AB, BC respectively ($p < 0.05$). The individual elements A and B and the interaction between BC variables ($p < 0.05$) were significant for the extraction of esculin, fraxin and esculetin. In the experiment, the content of fraxetin was low, indicating that there was no significant difference in the influence of variables on the design.

3.8. Method validation and sample analysis

To verify the reliability of the effervescence-assisted matrix solid-phase dispersion extraction, method validation covered a series of parameters including linear regression, detection limit, quantification limit and stability, and the results are summarized in Table 3. The RSD of retention times and chromatograms for the four analytes were all within 0.86%, demonstrating that the values were accurate and acceptable. By means of fitting chromatographic peak areas and solution concentration, the linear regression curves of the four analytes were $Y_{Esculin} = 5.4495\ x + 0.6826$ ($r^2 = 1$), $Y_{Fraxin} = 3.7348\ x + 0.2932$ ($r^2 = 1$), $Y_{Esculetin} = 8.8038\ x - 0.0099$ ($r^2 = 1$) and $Y_{Fraxetin} = 8.3011\ x - 0.3587$ ($r^2 = 1$). The results showed that the regression curves of the four analytes presented good linearity and the coefficients of determination were all 1. The LOD and LOQ ranged from 1.62 to 3.83 ng/mL and 5.39 to 12.76 ng/mL, respectively. These results demonstrated that acceptable accuracy, high sensitivity and optimized research of the proposed method were reliable. To enhance the applicability of this method, a recovery study was carried out by a standard-addition method for the assessment of the extraction method under optimal conditions. The results are shown in Table 4. Detailed data indicated that the recoveries were in the range of 91.37%–100.29% at the two concentration levels. Three samples were continuously prepared under optimal conditions for UHPLC analysis to investigate the reproducibility of the method. The results demonstrated that the repeatability was good and acceptable change (RSD less than 4.58%) was observed in retention time and chromatographic peak areas. The results of investigations on recovery and reproducibility indicated that the method has reliable precision and accuracy. The chromatograms are shown in Fig. 5. The peak area of the sample is comparable to that of methanol as the extraction solvent, but the method is more sensitive and environmentally friendly.

3.9. Comparison with other extraction methods

The proposed method was compared with other methods for the extraction of coumarin from plants and herbs. The extraction methods which were compared include UAE, MAE and ILSMP-UMSE, and the results are shown in Table 5. UAE is a conventional extraction method which can achieve good extraction efficiency and requires less sample, whether detected by CE or LC–MS/MS (Chen et al., 2009; Yun et al., 2011). However, in comparison its performance, there were apparent disadvantages in the consumption of extraction time and solvent. Considering the expenditure of 30 min and 45 min extraction times and the use of methanol, a toxic organic reagent, as the extraction solvent, it is unsuited for the desired rapid and environmentally friendly quantitative analysis. The MAE method had higher energy consumption to UAEbut with less than half the extraction time and the use of a nontoxic reagent, namely, PEG-200 (Zhou et al., 2011). Due to the extensive solubility of PEG-200 solution, the chromatogram was much more impure than the extraction solution of methanol. The ILSMP-UMSE method was characterized by its higher extraction efficiency and good recovery but had low sensitivity (Liu et al., 2015). Compared with the conventional extraction method, the EA-MSPD could use SDS as a green extraction solvent with a decrease in extraction time and sample consumption. This method simplified the extraction process and required no external energy consumption. Taking all of the factors above into consideration, EA-MSPD is a rapid and environmentally friendly analysis method for extraction of coumarins from *C. fraxini*.

4. Conclusion

In this research, a simple, rapid, environmentally friendly extraction method, termed effervescence-assisted matrix solid-phase dispersion, was exhibited and evaluated. By means of the dispersive force of CO_2 from effervescent tablets, the extraction solvent could be homogeneously dispersed into purified water which improved the mass-transfer efficiency. The optimal extraction conditions were as follows: effervescent salt, 100 mg sodium bicarbonate and 200 mg sodium dihydrogenphosphate; absorbent, 25 mg benzo-15-crown-5; extraction solvent, 100 mM SDS. Furthermore, the proposed method could transform hydrophobic compounds in aqueous solution and simultaneously determine the content of four coumarins. Moreover, no toxic organic solvents were used in EA-MSPD making it therefore more environmentally friendly and diffusely applied. Finally, the introduced method had been analyzed considering sensitivity, precision and recovery. The results indicate that the EA-MSPD extraction method presents high recovery, sensitivity, selectivity and low sample depletion for hydrophobic compounds. The method was successful in extracting four kinds of coumarin from *C. fraxini* and can be further explored in food analysis and environmental analysis.

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