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Microwave-assisted extraction in combination with capillary electrophoresis for rapid determination of isoquinoline alkaloids in *Chelidonium majus* L.

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ABSTRACT

A simple and rapid method based on microwave-assisted extraction (MAE) followed by capillary electrophoresis (CE) was developed for the quantification of eight isoquinoline alkaloids in *Chelidonium majus* L. (*Ch. majus*). The key parameters affecting CE separation and MAE extraction were investigated and optimized. Complete separation of eight alkaloids was achieved within only 9 min using a 500 mM Tris–H_3PO_4 buffer (pH 2.5) containing 50% (v/v) methanol and 2 mM HP-β-cyclodextrin. The optimal MAE extraction was performed at 60 °C for 5 min with methanol–water–HCl (90:10:0.5, v/v/v) as the extracting solvent, which gave much higher extraction efficiency in significantly shorter time than conventional heat reflux extraction (HRE) and ultrasonic extraction (USE) methods. Good linearities were obtained for all the alkaloids investigated with correlation coefficients above 0.9994. The repeatability and intermediate precision were less than 4.11% and the recoveries ranged from 98.0% to 103.9%. The developed method was successfully applied to 14 *Ch. majus* samples obtained from different regions of China. Compared with previously reported methods, the present method offers a dramatic savings in overall analysis time and considerable reduction in solvent consumption.

1. Introduction

Chelidonium majus L. (*Ch. majus*), also known by the common names "greater celandine" and "tetterwort", is one of the most important medicinal plants of the family *Papaveraceae*. Modern research has revealed that *Ch. majus* mainly contains a range of isoquinoline alkaloids, including sanguinarine, chelidonine, coptisine, protopine, stylopine, chelerythrine, berberine and allocryptopine, etc. (Fig. 1) [1]. Due to the presence of these alkaloids, the extracts of *Ch. majus* have been reported to possess various pharmacological activities, such as anti-inflammatory, antimicrobial, antiviral, anti-tumoral and cytotoxic effects [2–4]. Consequently, isoquinoline alkaloids have been considered as indices for estimation of quality of *Ch. majus*.

Some high-performance liquid chromatography (HPLC) methods [5–10] have previously been reported for the determination of isoquinoline alkaloids in *Ch. majus*. However, these methods suffered from several disadvantages, such as time-consuming sample preparation steps, large solvent consumption and long analysis time. Recently, Gu et al. [11] developed an ultra-performance liquid chromatography (UPLC) method for the quantification of seven main alkaloids in *Ch. majus*. Although this method showed some advantages including reduced run time, increased peak capacities and less solvent consumption over conventional HPLC, it required tedious sample preparation (heat reflux extraction followed by solid-phase extraction) prior to UPLC analysis.

Capillary electrophoresis (CE) is one of the most powerful tools for natural products analysis [12–14]. With the advantages of high separation efficiency, short analysis time and low reagent consumption, CE has been the focus of attention for developing new analytical methodology. Several papers have reported the use of CE to determine the main alkaloids in *Ch. majus* [1–3,15]. Kulp et al. [16] has most recently developed a CE method for the determination of seven isoquinoline alkaloids in *Ch. majus* with ultraviolet light-emitting diode-induced native fluorescence (UV-LEDIF) detection. In spite of the enhanced selectivity and sensitivity offered by the combination of CE and UV-LEDIF, application of this method is hampered by laborious sample preparation (ultrasonic extraction for 6 times followed by centrifugation, evaporation and reconstitution) and long analysis time.

In recent years, microwave-assisted extraction (MAE) has been proven to be a powerful sample extraction technique due to its ability to reduce the volume of extraction solvents and extraction time, improve the reproducibility and recovery of analytes and increase sample throughput [17–20]. MAE has been successfully applied to the extraction of some bioactive constituents in traditional Chinese medicines (TCMs) [21–24]. In the present work, the superiority of MAE over conventional extraction techniques for the

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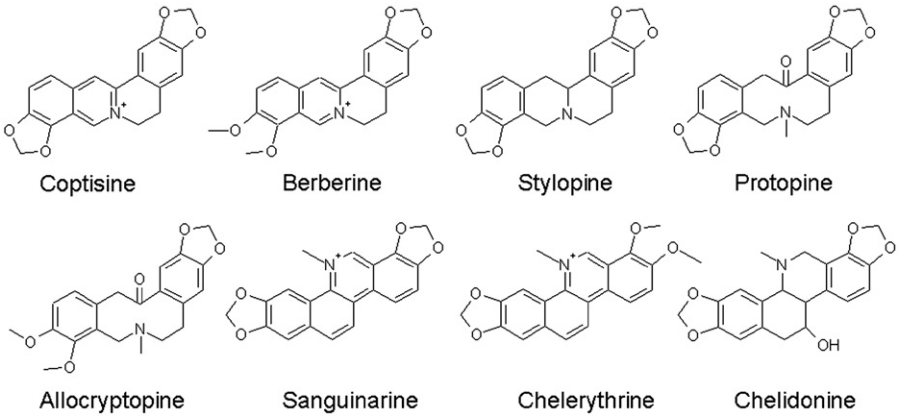

Fig. 1. Chemical structures of eight isoquinoline alkaloids from *Ch. majus*.

extraction of isoquinoline alkaloids from *Ch. majus* was investigated and demonstrated. For the first time, a simple and rapid method based on MAE followed by CE with photodiode-array detection was developed for the simultaneous determination of eight isoquinoline alkaloids in *Ch. majus*. The analytical advantages of the present method over previously published methods in terms of extraction time, analysis time and solvent consumption were illustrated.

2. Experimental

2.1. Reagents and materials

Sanguinarine (SAN), berberine (BER) and coptisine (COP) were purchased from Chengdu Mansite Pharmaceutical Co., Ltd. (Sichuan, China). Chelerythrine (CHE), chelidonine (CHD), protopine (PRO) and allocryptopine (ALL) were obtained from Shenzhen Medherb Biotechnology Co., Ltd. (Shenzhen, China). Stylopine (STY) was isolated and purified from *Ch. majus* in the Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University (Shenyang, China) and its chemical structure was confirmed based on UV, MS [m/z 324.1158($[M+H]^+$)], ^{1}H-NMR [600 MHz, $CDCl_3$: δ6.71(1H, s, H-1), 6.63(1H, s, H-4), 2.60–3.11(4H, m, H-5, 6), 3.51(1H, d, J=15.3 Hz, H8-α), 4.14(1H, d, J=15.3 Hz, H8-β), 6.65(1H, d, J=8.0 Hz, H-11), 6.63(1H, d, J=8.0 Hz, H-12), 2.83(1H, dd, J=15.6, 11.5 Hz, H13-α), 3.21(1H, dd, J=15.6, 11.5 Hz, H13-β), 3.59(1H, br.d, J=11.5 Hz, H-14), 5.94, 5.92(each 1H, d, J=1.4 Hz, 2,3-OCH_2O-), 5.91(2H, s, 9, 10 OCH_2O-)] and ^{13}C-NMR [150 MHz, $CDCl_3$: 104.1 (C-1), 145.2, 145.0(C-2, 3), 107.4(C-4), 126.4(C-4a), 29.3(C-5), 50.2 (C-6), 51.8(C-8), 116.2(C-8a), 141.3(C-9), 144.0(C-10), 106.5(C-11), 121.3(C-12), 130.2(C-12a), 36.3(C-13), 59.9(C-14), 127.1(C-14a), 100.9(2, 3-OCH_2O-), 101.6(9, 10-OCH_2O-)] data. The purities of all the compounds were over 98% determined by HPLC-UV. Sodium hydroxide, sodium borate, hydrochloric acid and phosphoric acid of analytical grade were obtained from Dongxing Corporation (Shenyang, China), Bodi Corporation (Tianjin, China), Xinyang Corporation (Henan, China) and Damao Corporation (Tianjin, China), respectively. Methanol, acetonitrile and isopropanol of HPLC grade were supplied by Concord Corporation (Tianjin, China). Tris ($>$99.8%) was from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Samples of *Ch. majus* were collected from different regions in China and were identified by Professor Qishi Sun, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University (Shenyang, China). The voucher specimens of these samples were deposited in the Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University (Shenyang, China).

2.2. Standard solutions

A mixed stock solution of SAN, BER, COP, CHE, CHD, PRO, ALL and STY was prepared in methanol. A series of working standard solutions were prepared by successive dilution of the stock solution with methanol. All the solutions were stored at 4 °C until use.

2.3. Extraction

2.3.1. Microwave-assisted extraction

MAE was performed with an Ethos A Microwave-assisted Extraction System (Milestone, Italy). 1.0 g of dried *Ch. majus* powder (20 mesh) was weighed accurately into a 100-mL Teflon extraction vessel and then extracted with 20 mL of methanol–water–HCl (90:10:0.5, v/v/v) for 5 min at 60 °C. After cooling, the extract was centrifuged at 12,000 rpm for 3 min and the supernatant was directly injected into the CE system.

2.3.2. Ultrasonic extraction

1.0 g of dried *Ch. majus* powder (20 mesh) was weighed accurately into a flask and then extracted with 20 mL of methanol–water–HCl (90:10:0.5, v/v/v) in an ultrasonic bath for 30 min. After cooling, the extract was centrifuged at 12,000 rpm for 3 min and the supernatant was directly injected into the CE system.

2.3.3. Heat reflux extraction

1.0 g of dried *Ch. majus* power (20 mesh) was weighed accurately into a 50-mL round bottom flask and then extracted with 20 mL of methanol–water–HCl (90:10:0.5, v/v/v) under reflux for 1 h in a water bath at 60 °C. After cooling, the extract was centrifuged at 12,000 rpm for 3 min and the supernatant was directly injected into the CE system.

2.4. CE analysis

CE analyses were carried out using an Agilent HP^{3D} Capillary Electrophoresis System (Agilent Technologies, Waldbrom, Germany) equipped with a built-in photodiode-array detector and controlled by the ChemStation CE software. Separation was performed on an uncoated fused-silica capillary (Yongnian Optic Fiber Plant, Hebei, China) of 50 μm i.d. and 375 μm o.d. with a total length of 35 cm (26.7 cm length to detection window). The temperature of the capillary cassette was maintained at 20 °C. The running buffer was 500 mM Tris–H_3PO_4 buffer (pH 2.5) containing 50% (v/v) methanol

and 2 mM HP-β-cyclodextrin. The separation voltage was held at 20 kV. The detection wavelength was set at 205 nm. Samples were loaded by pressure injection at 50 mbar for 5 s. Every day before the first run, the capillary was consecutively flushed with 0.1 M NaOH for 10 min, water for 10 min and the running buffer for 10 min. Between runs, the capillary was rinsed with 0.1 M NaOH, water and the running buffer for 3 min each.

3. Results and discussion

3.1. Optimization of CE conditions

Kulp et al. [16] reported that complete separation of seven isoquinoline alkaloids in *Ch. majus* was achieved within 22 min using an acidic phosphate buffer. In the present study, the possibility for achieving even faster CE separation of eight isoquinoline alkaloids was investigated. According to the theory of electrophoresis, it is possible to obtain increases both in speed and efficiency by increasing the voltage [25]. At the same time, the effects of Joule heating, which increases with increasing voltage, on efficiency should be considered. Several types of buffers including borate buffer, phosphate buffer and Tris–H_3PO_4 buffer were attempted in the separation of isoquinoline alkaloids. Finally, Tris–H_3PO_4 buffer was chosen as the running buffer, which has lower conductivity, allowing higher voltages and better separation efficiency. In the following study, the effects of buffer pH and concentration, organic additive and HP-β-cyclodextrin concentration on separation efficiency were investigated.

3.1.1. Effects of buffer pH and concentration

We initially investigated the migration behaviors of eight alkaloids in the range of pH 2.0–6.0. It was found that SAN, COP and CHE had much higher migration velocities than others, mainly due to their relatively strong basic properties [16]. At a pH higher than 2.7, severe peak tailing was observed for SAN and CHE. This phenomenon has been explained by the pH-dependencies of SAN and CHE solubility [26]. Therefore, a pH optimization was carried out within the range of 2.0–2.7. Fig. 2 shows the effect of pH on the resolution of adjacent peak pairs. It can be seen that peak pairs of CHE-U_1 (U_1 is a later-migrating peak near to CHE), BER-U_2 (U_2 is a later-migrating peak near to BER) and ALL-STY are the three critical pairs that are difficult to separate. The resolution of peak pair of ALL-STY increased with the increase of pH, but meanwhile the resolution of peak pairs of CHE-U_1 and BER-U_2 decreased. The compromising result was achieved at pH 2.5. Subsequently, the concentration of Tris–H_3PO_4 buffer in the range of 200–500 mM was optimized at the optimum buffer pH of 2.5. With the increase of buffer concentration, the migration times were prolonged and the separation efficiency was increased. When the buffer concentration was higher than 500 mM, excessive current was observed. As a result, 500 mM of Tris–H_3PO_4 buffer was chosen as the optimum buffer concentration.

3.1.2. Effects of organic additive and HP-β-cyclodextrin concentration

As most of the isoquinoline alkaloids in *Ch. majus* have low solubility in aqueous media, addition of an organic modifier to the running buffer has been reported to increase the solubility and enhance the separation efficiency. Different organic additives including methanol, acetonitrile and isopropanol were studied. Precipitation was found when a high percentage of acetonitrile was added to 500 mM Tris–H_3PO_4 buffer. Addition of isopropanol had a negative effect on the separation efficiency. So methanol was chosen as an organic additive and its percentage was optimized in the range of 40% to 60%. It was found that a relatively low percentage of methanol could not give satisfactory resolution, peak shape and migration time. But when a very high methanol percentage was used, precipitation of the buffer appeared. Finally, 50% methanol was chosen as the optimum organic modifier.

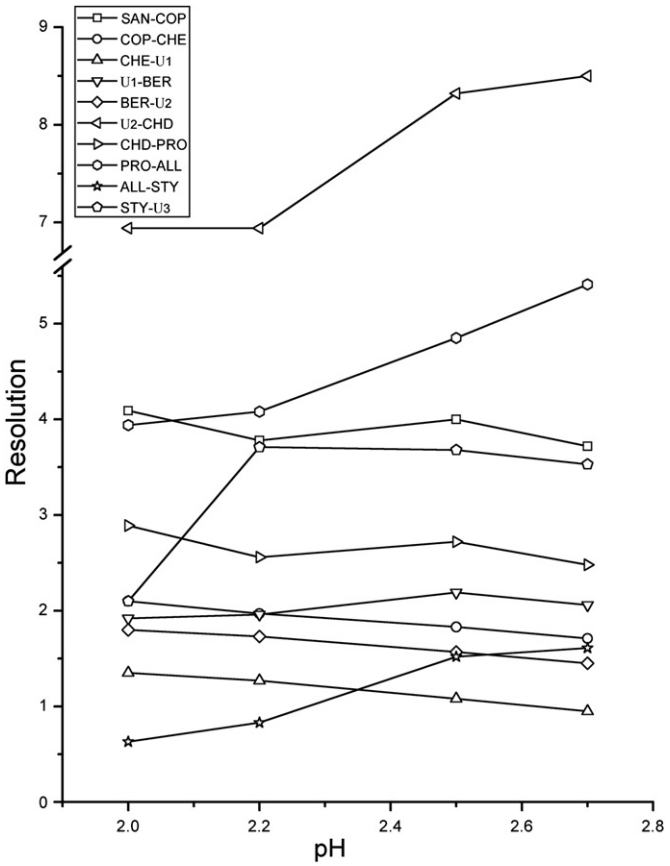

Fig. 2. Effect of buffer pH on the resolution of adjacent peak pairs. U_1, U_2 and U_3 are unknown components in *Ch. majus*.

Using a 500 mM Tris–H_3PO_4 buffer (pH 2.5) containing 50% methanol, complete separation of the eight alkaloids in a standard mixture was achieved. However, such a condition was not suitable for real application, since the peak pair of CHE-U_1 was not well separated. To improve the resolution of this peak pair, addition of different concentrations of HP-β-cyclodextrin (0.5–5 mM) to the buffer was attempted. The results showed that the resolution of peak pair of CHE-U_1 increased with the increase of HP-β-cyclodextrin concentration, but meanwhile the resolution of peak pair of COP-CHE decreased. The best compromise was obtained with 2 mM HP-β-cyclodextrin.

Under the above optimum conditions, electropherograms of a standard mixture of eight alkaloids (A) and an extract from a *Ch. majus* sample (B) are shown in Fig. 3.

3.2. Optimization of MAE conditions

The effects of extraction solvent, temperature, time and solvent to material ratio on the extraction efficiency of eight alkaloids were investigated systematically under different MAE conditions, followed by CE analysis [27]. The areas of CE peaks were used for the comparison of MAE results.

3.2.1. Selection of solvent in MAE

Selection of an appropriate solvent is fundamental to obtaining an optimal MAE condition [20]. For the present work, different

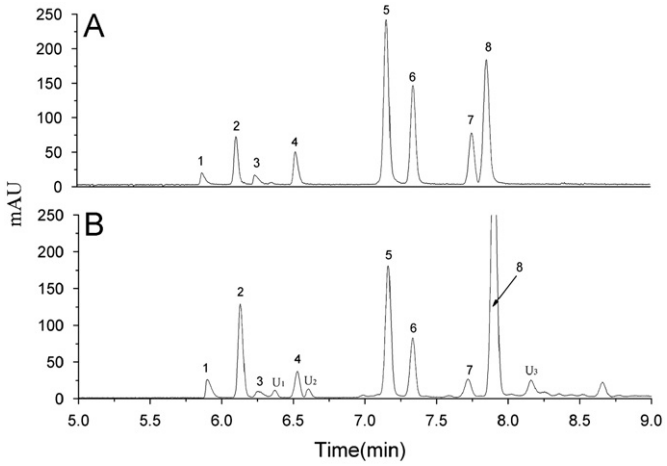

Fig. 3. Electropherograms of a standard mixture of eight alkaloids (A) and an extract from a *Ch. majus* sample (B). Peaks: (1) sanguinarine; (2) coptisine; (3) chelerythrine; (4) berberine; (5) chelidonine; (6) protopine; (7) allocryptopine; (8) stylopine; U_1, U_2 and U_3 are unknown components in *Ch. majus*. Buffer: 500 mM Tris–H_3PO_4 buffer (pH 2.5) containing 50% (v/v) methanol and 2 mM HP-β-cyclodextrin. Separation capillary: 50 μm i.d. uncoated fused-silica capillary, 35 cm in length (26.7 cm effective length). Applied voltage: 20 kV. Detection wavelength: 205 nm.

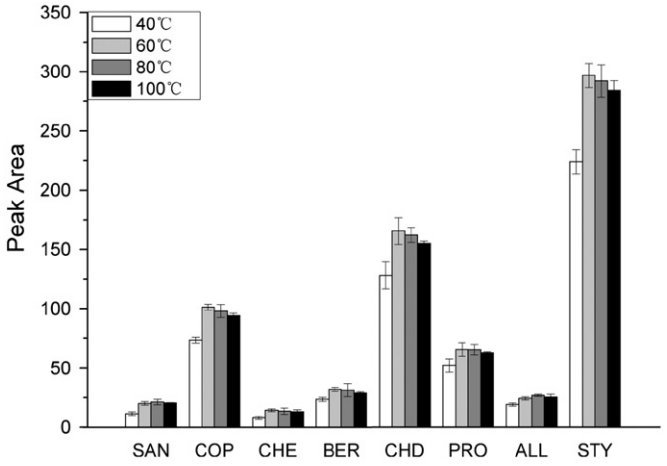

Fig. 4. Effect of temperature on the extraction of eight alkaloids from *Ch. majus* ($n=3$).

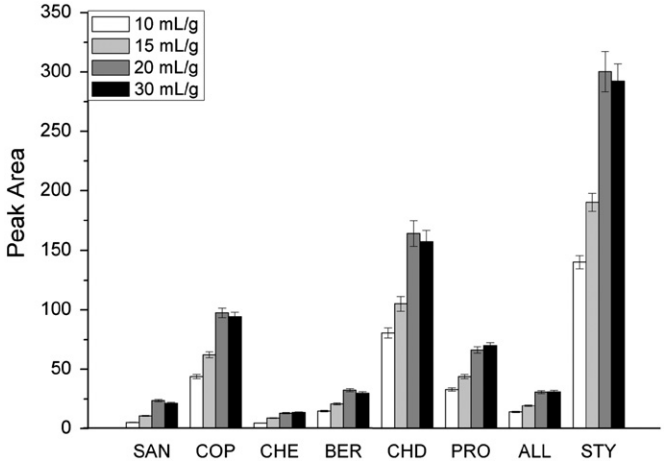

Fig. 5. Effect of solvent to material ratio on the extraction of eight alkaloids from *Ch. majus* ($n=3$).

extraction solvents including methanol, ethanol, water, methanol–water (90:10, 70:30, 50:50, v/v) and ethanol–water (90:10, 70:30, 50:50, v/v) were tested with MAE under the same condition. It was found that methanol–water (90:10, v/v) gave the highest extraction efficiency. As the isoquinoline alkaloids are weak bases, the addition of small quantities of acid to the extraction solvent can improve the solubility of alkaloids [28]. Different percentages of hydrochloric acid (0, 0.5%, 1%, 2%) in methanol–water (90:10, v/v) were further tested. The results showed that adding some acid to the solvent could significantly enhance the extract efficiency of eight alkaloids. But the percentage of hydrochloric acid in the solvent was found to have negligible effect on the MAE results. Hence, methanol–water–HCl (90:10:0.5, v/v/v) was selected for further experiments.

3.2.2. *Effects of extraction temperature and time*

Extraction temperature is a key factor in the optimization of MAE procedure [29]. In order to investigate the effect of temperature on the extraction efficiency of eight alkaloids from *Ch. majus*, four different temperatures ranging from 40 to 100 °C were examined. As shown in Fig. 4, the extraction efficiency of the alkaloids was improved at elevated temperatures since increasing temperature will enhance solvent diffusivity, reduce surface tension and increase solute solubility. However, at temperatures over 60 °C, a slight decrease of some peak areas were observed, perhaps as a result of thermal degradation of some alkaloids. Hence, an extraction temperature of 60 °C was selected for further experiments.

The effect of extraction time on extraction efficiency was studied while maintaining the extraction temperature at 60 °C. Extraction times of 5, 10 and 30 min were examined. The results showed that no significant increase in peak area was obtained with the increase of the extraction time (figure not shown). Therefore, 5 min was chosen as the optimal time for MAE.

3.2.3. *Effect of solvent to material ratio*

Using an extraction temperature of 60 °C and an extraction time of 5 min, the effect of solvent to material ratio on extraction efficiency was investigated. Extraction was carried out at four different ratios of solvent to material (10, 15, 20 and 30 mL/g). It can be seen from Fig. 5 that the normalized peak areas of the alkaloids increased with the increase of solvent to material ratio and reached the highest at 20 mL/g. Then most of the peak areas decreased slightly, perhaps due to inadequate stirring of the solvent by microwaves. Therefore, 20 mL/g was considered as the optimal ratio of solvent to material for the MAE process.

Based on the above findings, the optimal MAE condition for the extraction of eight isoquinoline alkaloids in *Ch. majus* was: 1.0 g of sample, 20 mL of methanol–water–HCl (90:10:0.5, v/v/v), 60 °C and 5 min. It should be noted that repeated extraction did not give significantly higher extraction efficiency than a single extraction. So in view of saving time and energy, one-step extraction was enough to extract the alkaloids from *Ch. majus*.

The optimal MAE method was compared with two conventional extraction methods, namely HRE and USE. The results are presented in Fig. 6. It can be seen that MAE gave significantly higher extraction efficiency than HRE and USE. Moreover, MAE took much less extraction time (only 5 min) than HRE (1 h) and USE (30 min). With the capability of parallel processing of multiple sample (10 samples in a single batch), MAE is much more attractive for high-throughput sample preparation.

3.3. *Method validation*

3.3.1. *Linearity*

The linearity of the method was evaluated by injecting a series of standard solutions in triplicate at six concentration levels.

Calibration curves were constructed by non-weighted least squares linear regression analysis of peak area (y) of the analyte versus the nominal concentration (x). An analysis of variance (ANOVA) was performed to test the significance of the regression. The lack-of-fit (LOF) test was used to determine whether the selected model is adequate to describe the experimental data. The results of regression analysis are summarized in Table 1. Good correlations were obtained for all the analytes with r values higher than 0.9994. The p-values for LOF test are greater than 0.05 for all the analytes, indicating that the linear regression models are adequate for the experimental data.

3.3.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were estimated as 3 and 10 times the signal-to-noise ratio (S/N), respectively. The LOQs of eight analytes were 2.15–14.15 μg/mL and the LODs were 0.65–4.28 μg/mL (Table 1). Although the LODs for some alkaloids are an order of magnitude higher than those determined by CE with UV-LEDIF detection, the limits are low enough to determine the presence of all the alkaloids under consideration in real *Ch. majus* samples.

3.3.3. Precision

The injection precision was determined by 10 subsequent injections of a standard solution. The relative standard deviations (RSDs) of migration time and peak area were in the range of 0.23–0.54% and 0.95–3.11%, respectively. The repeatability of the method was assessed by six replicate analyses of the same *Ch. majus* sample. The RSDs of migration time and peak area were all below 4.11% (Table 2). Intermediate precision was examined similarly to that of repeatability but by two analysts, on 3 consecutive days. As shown in Table 2, the RSDs ranged from 0.20% to 4.00%. The results indicate an acceptable level of precision.

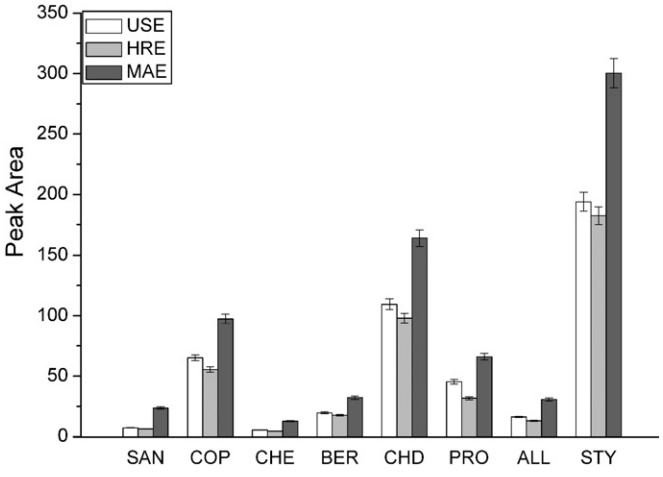

Fig. 6. Comparison of MAE with conventional extraction techniques (n=3).

3.3.4. Accuracy

The accuracy of the method was determined by performing spike and recovery experiments. Six replicate samples were prepared by spiking known amounts of standards to a real *Ch. majus* sample. Percentage recoveries were calculated by comparison of found and added amounts of the analytes in spiked samples. The original amounts of the analytes in the sample were subtracted from the measured amounts of each spiked sample before calculating recoveries. The recoveries of the eight alkaloids ranged from 98.0% to 103.9% (Table 2), demonstrating good reliability of the method for analysis of eight alkaloids in *Ch. majus*.

3.3.5. Stability

The stability of the analytes in the final extract stored at room temperature was investigated by replicate injections of a freshly prepared sample solution at 2-h interval up to 8 h. The RSDs of the assay results were 2.44–4.42%, which indicate that the sample solution is stable at room temperature for at least 8 h.

3.3.6. Robustness

The robustness of the developed method was evaluated by making deliberate variations in CE parameters such as capillary temperature, buffer pH, Tris concentration and separation voltage. The parameters were varied 5% below and above the value set in the method. Resolutions of peak pairs of COP-CHE, CHE-U_1, BER-U_2 and ALL-STY (four critical pairs) were used as indicators. No significant change was observed through variations of capillary temperature and separation voltage. Variations in buffer pH and Tris concentration resulted in variations in resolution of about 15%, indicating that these two parameters need to be closely controlled. The effect of capillary on resolution was studied using two different batches of capillaries. No significant variations were observed regarding the resolution of four critical pairs.

3.4. Real sample analysis

The proposed analytical method was applied to analysis of 14 batches of *Ch. majus* samples obtained from different regions of China. The quantitative results were summarized in Table 3. It can be seen that COP was the most abundant alkaloids in all *Ch. majus* samples. The contents of ALL and CHE were significantly lower than those of the other alkaloids in all samples. Due to the differences in the region of cultivation and harvesting time, the contents of eight alkaloids in *Ch. majus* varied greatly from sample to sample.

3.5. Comparison with previously reported methods

Comparison of the proposed method with some published analytical methods for the determination of isoquinoline alkaloids in *Ch. majus* was summarized in Table 4. It can be seen that the MAE approach significantly reduced the sample preparation time

Table 1
Linearity, LOD and LOQ for quantification of eight alkaloids in *Ch. majus*.

Compound	Linear range (μg/mL)	Regression equation	r	F_{LOF}	LOQ (μg/mL)	LOD (μg/mL)
Sanguinarine	14.1–450.0	$y=0.397x+1.224$	0.9996	$0.343(p>0.05)$	14.10	4.27
Coptisine	28.3–904.0	$y=0.702x+8.491$	0.9995	$1.325(p>0.05)$	14.15	4.28
Chelerythrine	6.2–198.4	$y=0.617x+9.840$	0.9998	$1.969(p>0.05)$	6.20	1.87
Berberine	12.5–400.0	$y=2.063x-8.823$	0.9994	$2.863(p>0.05)$	12.50	3.78
Chelidonine	22.8–728.0	$y=3.658x-0.586$	1.0000	$1.740(p>0.05)$	2.28	0.69
Protopine	12.3–394.0	$y=4.168x-2.013$	0.9996	$3.761(p>0.05)$	2.50	0.75
Allocryptopine	5.0–160.8	$y=9.083x-11.14$	0.9994	$4.011(p>0.05)$	2.15	0.65
Stylopine	12.3–394.0	$y=6.185x-6.210$	0.9995	$0.928(p>0.05)$	2.40	0.90

Table 2
Precision and accuracy for quantification of eight alkaloids in *Ch. majus*.

Compound	Injection precision RSD (%)		Repeatability RSD (%)		Intermediate precision RSD (%)		Recovery (%)
	Peak area	Migration time	Peak area	Migration time	Peak area	Migration time	
Sanguinarine	2.05	0.23	2.91	0.20	4.00	0.21	99.9 ± 3.0
Coptisine	2.20	0.25	2.23	0.21	2.86	0.23	100.2 ± 2.9
Chelerythrine	2.49	0.25	4.11	0.21	3.06	0.20	99.5 ± 4.8
Berberine	2.47	0.26	2.88	0.21	3.17	0.22	101.8 ± 4.1
Chelidonine	3.11	0.24	2.24	0.22	3.30	0.23	100.7 ± 3.4
Protopine	1.50	0.25	1.74	0.21	2.53	0.23	98.0 ± 2.8
Allocryptopine	0.95	0.26	3.57	0.21	2.92	0.22	103.9 ± 3.7
Stylopine	1.17	0.54	3.46	0.30	2.65	0.28	100.0 ± 2.7

Table 3
Contents of eight alkaloids (mg/kg) in 14 batches of *Ch. majus* samples ($n=3$).

Sample	Sanguinarine	Coptisine	Chelerythrine	Berberine	Chelidonine	Protopine	Allocryptopine	Stylopine
Liaoning1	1080 ± 80	5768 ± 305	338 ± 13	1161 ± 50	864 ± 4	833 ± 38	157 ± 7	2347 ± 102
Hebei2	1122 ± 19	2933 ± 312	384 ± 2	517 ± 5	977 ± 12	511 ± 3	110 ± 4	1127 ± 17
Heilongjiang1	1101 ± 10	3524 ± 25	437 ± 13	505 ± 9	798 ± 19	522 ± 3	150 ± 9	2026 ± 104
Sichuan1	1760 ± 23	8799 ± 375	783 ± 21	902 ± 25	1008 ± 27	876 ± 10	208 ± 6	1150 ± 37
Liaoning2	909 ± 2	4138 ± 9	294 ± 1	908 ± 2	687 ± 4	678 ± 4	120 ± 5	1796 ± 23
Zhejiang	1127 ± 39	6700 ± 218	476 ± 20	500 ± 21	719 ± 14	599 ± 6	175 ± 4	1159 ± 61
Jilin1	1391 ± 31	7408 ± 1103	459 ± 4	682 ± 14	885 ± 19	700 ± 23	209 ± 10	1118 ± 18
Sichuan2	1456 ± 42	8045 ± 50	635 ± 15	634 ± 24	851 ± 35	748 ± 20	194 ± 10	1617 ± 68
Heilongjiang2	1183 ± 14	2804 ± 61	445 ± 12	531 ± 10	886 ± 11	448 ± 4	127 ± 2	2069 ± 80
Shanxi	1224 ± 40	6227 ± 122	420 ± 2	541 ± 4	722 ± 37	566 ± 20	150 ± 3	1873 ± 3
Jiangsu	852 ± 6	4974 ± 186	298 ± 3	410 ± 7	514 ± 12	475 ± 9	134 ± 3	1275 ± 18
Hernan	1144 ± 16	5988 ± 229	403 ± 9	501 ± 14	670 ± 30	560 ± 24	165 ± 3	1704 ± 24
Jilin2	1319 ± 12	6029 ± 145	426 ± 16	550 ± 2	793 ± 29	634 ± 24	157 ± 2	1627 ± 13
Hebei1	1209 ± 41	3209 ± 120	432 ± 16	499 ± 4	1012 ± 36	542 ± 11	127 ± 6	1674 ± 27

Table 4
Comparison of different methods for quantification of alkaloids in *Ch. majus*.

Sample preparation				Sample analysis				References
Extraction method[a]	Subsequent sample treatment	Time	Solvent consumption (mL/g sample)	Method	Time	Number of analytes	Solvent consumption (mL per run)	
MAE	Centrifugation	8 min	20	CE	9 min	8	$<10^{-5}$	Current
IE and USE	Laying aside	12.5 h	10	HPLC	50 min	8	50	[6]
USE	Centrifugation, evaporation, SPE	1.5 h	600	HPLC	40 min	5	40	[9]
IE	Evaporation	4 weeks	360	HPLC	25 min	7	12.5	[10]
HRE	SPE, filtration	1.5 h	30	UPLC	20 min	7	8	[11]
USE	Centrifugation, evaporation, reconstitution	1 h	240	CE	24 min	7	$<10^{-5}$	[16]

[a] MAE, microwave-assisted extraction; IE, immerse extraction; USE, ultrasonic extraction; SPE, solid-phase extraction; HRE, heat reflux extraction.

from several hours to 8 min and the analysis time of the present CE method was 2–6 times shorter than those reported in the literature. Moreover, considerable reduction of solvent consumption was achieved using the present method. These results indicate that MAE combined with CE analysis is a good choice for rapid extraction and analysis of isoquinoline alkaloids in *Ch. majus*.

4. Conclusions

A simple, fast and reliable method based on MAE combined with CE analysis was developed and validated for the simultaneous determination of eight isoquinoline alkaloids in *Ch. majus* for the first time. The developed method was successfully applied to determine eight alkaloids in 14 batches of *Ch. majus* obtained from different regions of China. In comparison with previously published methods, the present method offers a dramatic savings in sample analysis time and solvent consumption. The combination of MAE with CE was an effective method for rapid analysis of isoquinoline alkaloids in *Ch. majus*.

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