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Research Article

Novel approach to improve the detection of colchicine via online coupling of ionic liquid-based single-drop microextraction with capillary electrophoresis

A novel approach based on ionic liquid-single-drop microextraction (IL-SDME) online coupling with capillary electrophoresis (CE) was used to determine a toxic alkaloid – colchicine. The IL-SDME procedure was optimized by extraction solvent, drop volume controlling, sample volume and pH, extraction time, and ionic strength. Under optimum conditions, enrichment factor was as much as 41-fold with a relative standard deviation of 2.8% ($n = 3$). Linear range of response was observed from 1 to 100 μg/mL, with detection limit of 0.25 μg/mL and correlation coefficient (R^2) of 0.9994. The extraction of colchicine from spiked Lanzhou lily sample was performed and obtaining good result with an average recovery rate of 102.4 and 98.8% at 5 and 50 μg/mL, respectively. Comparing with the previous methods, IL-SDME-CE is really a convenient, economical, and environmentally benign way for determining colchicine.

Keywords: Capillary electrophoresis / Colchicine / Ionic liquid / Online coupling / Single-drop microextraction
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1 Introduction

Colchicine is a naturally occurring alkaloid of plant origin used in human medicine [1]. The molecular structure of colchicine is shown in Fig. 1. It is poisonous in *in vitro* and *in vivo* systems even at low concentrations. The toxicity of colchicine for human has been known ever since the use of plant extraction; however, there are only a few literatures in which colchicine levels were monitored [2–4]. With the development of analytical technology, the detection of colchicine by various methods had been established. A monolithic reversed-phase column for separation of related alkaloids from colchicine in dry extract from colchicum seeds was reported [5]. Bodoki et al. [6] proposed the use of a densitometric determination for colchicine from pharmaceutical products and seeds of meadow saffron. The determination of colchicine by electrochemistry and voltammetric determination was also proposed and applied to human urine samples [7, 8]. A sensitive and rapid method to determine colchicine in human plasma by liquid chromatography-mass spectrometry (LC-MS) had been developed and was applied to a pharmacokinetic study of colchicine [9]. However, there were only two reports about colchicine detection on capillary electrophoresis (CE) which reported the separation and detection of four toxic alkaloids including colchicine on microchip-based CE with detection limit (LOD) of 2.4 mg/L [10] and developed an extraction, separation, and detection method for colchicine in beverages on CE with LOD of 1.1 μM [11]. That may be because the low detection sensitivity of CE results from small injection volume of sample and short optical path length, which limits the application of CE for the analysis of many target analytes [12]. Thus, it is necessary to couple the sample preparation with CE to enhance the sensitivity for real sample analysis [13].

Recently, there is a strong trend toward the miniaturization of chemical analysis systems since they have several distinct advantages (e.g. fast analysis, small sample volume, and portability). In addition, an environmentally friendly feature of the miniaturized analysis system is to reduce the consumption of reagents. Therefore, liquid-phase microextraction (LPME) emerges because of demand. Additionally, CE is an attractive analytical technique with advantages such as fast analysis, simple operation, and low running cost. Thus, the method combining LPME and CE is prospective and is performed with extremely small amounts of reagents and solvents [14]. An analytical technique of inline coupling headspace LPME with CE was reported to determine volatile analytes [15].

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Abbreviations: EF, enrichment factor; **IL,** ionic liquid; **LPME,** liquid-phase microextraction; **SDME,** single-drop mictroextraction

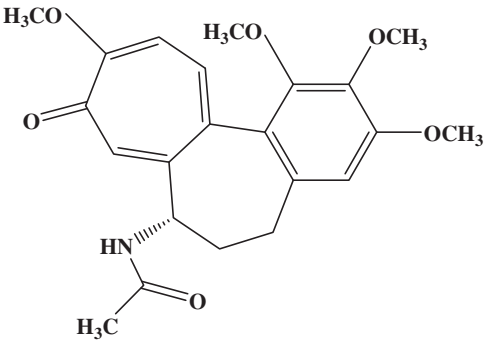

Figure 1. Molecular structure of colchicine.

Single-drop microextraction (SDME) is a miniaturization of traditional liquid–liquid extraction (LLE) technique, whereby a microliter drop of a water immiscible organic solvent is exposed to the sample solution, achieving preconcentration of target compounds in a simple and fast procedure. As a good sample preparation method, SDME has been online coupled with CE successfully by Choi et al. in 2009 [16]. Then, they reported the coupling of SDME with large-volume stacking [17] and sweeping [18] on CE which made the method more perfect and sensitive. After that, more and more studies were done about online coupling of SDME and CE. Online SDME-CE method was applied to concentrate and detect target analytes, such as phenols [15], adenine [19], amino acids, and peptides [20]. In these researches, extraction solvents were organic solvents which might have effects on environment because evaporation of the extracting solvent drop was an important drawback that deteriorated the method figures of merit.

On the other hand, ionic liquids (ILs) are found more suitable to be used as extractants in SDME than common organic solvents [21]. ILs are a group of new organic salts consisting of a combination of organic cations and various anions that are liquid at room temperature [22]. Important features of ILs include their immeasurably low vapor pressure, high stability and viscosity, moderate dissolvability of organic compounds as well as tailor-made miscibility, and polarity, which have made them as attractive acceptor phase for SDME in atomic absorption spectrometry [23], HPLC [24–29], or GC–MS [22, 30]. Most recently, we developed a novel online IL–SDME method for CE analysis of phenol model compounds, demonstrating IL as a benign SDME solvent for CE [31]. Based on these researches, the SDME-CE would be a prospective technique for real sample analysis.

To the best of our knowledge, there is no available data about the assessment for IL-SDME-CE applications in food sample in relative current reports. Meanwhile, Lanzhou lily is one kind of famous food materials in China. However, there is no report about the amount of colchicine in Lanzhou lily which is Chinese species of colchicum. In this article, online coupling of IL-SDME and CE was developed for the enhancement in detection sensitivity for colchicine. Parameters including extraction solvent, IL single-drop volume, extraction time, ionic strength, sample solution volume, and sample pH were optimized. Under the optimized conditions, the method was applied to the determination of colchicine in Lanzhou lily aqueous sample.

2 Materials and methods

2.1 Chemicals

Colchicine (HPLC grade) was purchased from Sigma-Aldrich (USA). Methanol was obtained from Tianjin Baishi Chemical Factory (Tianjin, China). Sodium chloride was obtained from Shanghai Fine Chemicals (Shanghai, China). Sodium tetraborate was from Baiyin Chemical Reagent Factory (Baiyin, China). The ILs of 1-alkyl-3-methylimidazolium hexafluorophosphate ($[C_nMIM][PF_6]$, $n = 4, 6, 8$) were supplied by Center for Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics (Lanzhou, China). All reagents were analytical grade unless otherwise stated. The Lanzhou lily sample was purchased from local supermarket.

2.2 Instrumentation

All experiments were carried out on an Agilent 3D CE system with a UV–vis detector (Agilent Technologies, Germany). An uncoated 75 μm fused silica capillary of total length 48.5 cm and effective length 40 cm (Ruifeng, Hebei) was used for analysis. Data acquisition was performed by ChemStation software. During analysis, a constant voltage of 20 kV was applied, and the temperature was maintained at 25°C. For all analysis, the wavelength of detection was set at 254 nm (IL has no absorbance at this wavelength) [32]. The final optimized running buffer consisted of 20 mM borate (pH 10.0) with 66.7% methanol (v/v). Prior to use, all solutions were filtered and ultrasonically degassed. Prior to analysis, a fresh capillary was conditioned with methanol (5 min), followed by distilled water (5 min), 1 M sodium hydroxide (30 min), distilled water (10 min), and finally running buffer (5 min) by flushing. At the beginning of each day, the capillary was flushed with distilled water, 1.0 M sodium hydroxide, distilled water for 5 min, respectively, in sequence. The capillary was flushed after each run with methanol for 2 min, followed by running buffer for 2 min. The injected volume of IL was calculated using the Beckman CE Expert Software (Generic CE mode). All experiments were performed at least in triplicate to ensure reliability of the data presented.

2.3 IL-SDME-CE procedure

The procedure of IL-SDME-CE was carried out according to our previous study [31]. Briefly, the capillary was flushed

with BGE for 2 min. Then, IL was injected into the capillary inlet under 945 mbar for 0.05 min, followed by applying pressure of −50 mbar for 110 s so that an IL single drop was formed at the tip of the capillary inlet and extraction process began. With a purpose of counteracting the surface force of the IL drop, a pressure of −6 mbar was applied for 1 min after every extraction for 2 min. After extraction, IL drop enriched with the target analyte was injected into the capillary under 50 mbar for 3 s and CE running began.

2.4 Standard and real sample preparation

The colchicine was dissolved in distilled water to obtain a standard stock solution with the concentration of 1000 μg/mL and then stored at 4°C. Working solutions were prepared daily by dilution of the stock colchicine solution with distilled water.

Briefly, for real sample preparation, 5.0 g fresh lily was accurately weighted. The lily sample was sliced into small pieces. After drying at room temperature, the sample was ground to powder and then used for extraction. The sample was extracted with 10 mL distilled water for 30 min in an ultrasonic bath and followed by centrifugation at 8000 rpm for 5 min. The supernatant was transferred to a volumetric flask. The extracting procedure was repeated two times. The total volume of extracts was filtered through a 0.45-μm micropore membrane and diluted with distilled water to a final volume of 25 mL with NaCl content of 30% w/v for direct analysis. The sample was stored in a refrigerator before analysis.

3 Results and discussion

3.1 Basic principle of IL-SDME

There are multiplicate forces between ILs and many compounds [33]. There is a π–π interaction between the imidazole ring of ILs and aromatic rings of the colchicine. Therefore, ILs may have good extraction efficiency for colchicine.

The enrichment factor (EF) was calculated as follows:

$$\mathrm{EF} = \frac{C_{\mathrm{IL}}}{C_{\mathrm{aq,i}}} \quad (1)$$

where C_{IL} and $C_{\mathrm{aq},i}$ represent the final concentration of colchicine in the IL phase after IL-SDME and the initial concentration of colchicine in the sample solution, respectively. C_{IL} was obtained by injection of IL phase after extraction and $C_{\mathrm{aq},i}$ was obtained by direct injection of the sample aqueous solution before extraction.

3.2 Optimized conditions of IL-SDME

To develop an IL-SDME-CE method for the determination of colchicine in real samples, several parameters controlling optimum extraction efficiency, including selection of ILs, effect of single drop volume, extraction time, ionic strength, sample volume, and sample pH were systematically investigated. All optimization experiments were performed with distilled water samples spiked with 50 μg/mL of the colchicine.

3.2.1 Selection of ILs

It is very important to select an appropriate extraction solvent in this method. It should have low volatility, proper viscosity, and hydrophobicity. ILs were chosen because of their unique characteristics. The effect of alkyl side chains of ILs on extraction efficiency was investigated. Three ILs, $[C_4MIM][PF_6]$, $[C_6MIM][PF_6]$, and $[C_8MIM][PF_6]$, were compared as extraction solvents. As can be seen from the experimental results, $[C_4MIM][PF_6]$ had the best extraction efficiency in three ILs. With the increasing of alkyl side chain length, the extraction efficiency was decreased. It is because that the longer the alkyl side chain is, the higher the viscosity of IL is [34]. Thus, it is difficult for the analyte to diffuse into $[C_6MIM][PF_6]$ and $[C_8MIM][PF_6]$ so that the extraction efficiencies are poor for these two ILs. Meanwhile, given more time to dissolve the $[C_6MIM][PF_6]$ and $[C_8MIM][PF_6]$ after injection, which has adverse influence in CE separation, $[C_4MIM][PF_6]$ was chosen as extraction solvent in the further experiments.

3.2.2 IL single-drop formation and optimization of single-drop volume

The volume of IL single drop is one of the key parameters of the IL-SDME-CE. In this proposed method, we controlled the formation of IL single drop by controlling the injection pressure and injection time. The procedure of IL single-drop formation has been elaborated in Section 2.3. It is well known that the volume of IL single drop has effect on extraction efficiency. We investigated the single-drop volume in the range from 2.40 to 9.60 nL by adjusting the IL injection time from 0.05 to 0.2 min at 945 mbar. The single-drop volume was calculated according to Poiseuille equation [35]. As shown in Fig. 2, the peak area was decreasing with the increasing of the volume of IL single drop. That is because the extracted colchicine in smaller volume of extraction solvent has higher concentration. Meantime, the bigger drop has bigger surface area so that the colchicine diffused to IL drop easier. As can be seen, the two processes simultaneously exist but the former is dominant. Additionally, it is difficult to reduce the volume of IL drop further since the single drop would become unstable and even disappear after a long extraction time because of dissolution into sample solution. In view of the good efficiency, 2.40 nL was chosen for subsequent experiments.

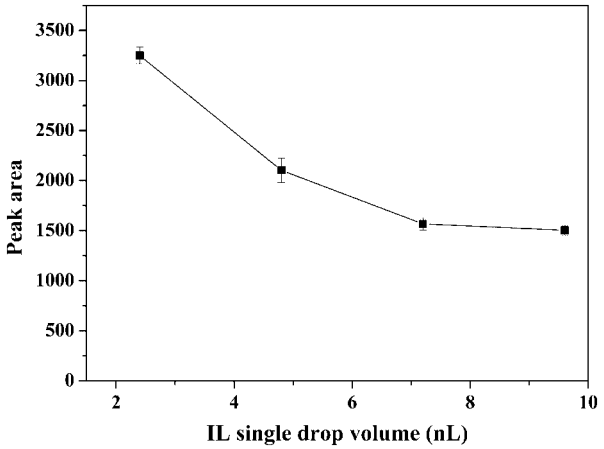

Figure 2. Effect of volume of IL single drop on peak area of colchicine extracted by the IL-SDME-CE. IL-SDME conditions: extraction time, 10 min; IL single-drop volume, 2.4 nL; sample volume, 600 μL; 30% NaCl (w/v); 25°C; colchicine concentration, 50 μg/mL. The error bars represent the standard deviation.

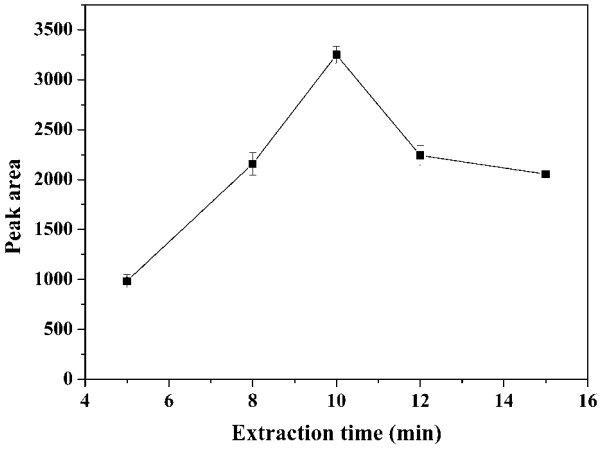

Figure 3. Effect of extraction time on peak area of colchicine extracted by the IL-SDME-CE. Other IL-SDME conditions are the same as in Fig. 2, except the extraction time. The error bars represent the standard deviation.

3.2.3 Effect of extraction time

Extraction time is an important parameter in extraction. In this method, extraction was performed with different extraction times in the range of 5–15 min. The results of the effect of extraction time on colchicine behavior are shown in Fig. 3. As shown in Fig. 3, with the increasing of extraction time, the peak area was increased, but the peak area was decreased after 10 min. The reason may be because the longer exposure time allows the colchicine to transfer to IL drop from aqueous sample sufficiently. However, IL may dissolve in sample solution to a certain extent [36], and the methanol in buffer may dissolve IL too. Thus, after a long extraction time, IL drop may become small so as to decrease the extraction efficiency. The probable reason for decreasing extraction efficiency by IL drop dissolution is that the extracted analytes may be concentrated on the exterior part of the drop in SDME. On the one hand, the analyte was diffused into the IL single drop; on the other hand, the outer regions of the drop were dissolved in sample solution. Additionally, the dissolution portion of IL drop in sample solution may have adverse effect on the distribution of the analyte in IL drop. The simultaneous processes resulted in the present behavior. Therefore, 10 min was applied as the optimal extraction time for further experiments.

3.2.4 Effect of ionic strength of sample solution

Salt is usually added to sample solution to increase ionic strength, and thus improving extraction efficiency. The effect of the content of NaCl on extraction efficiency is shown in Fig. 4. It was observed that an increase in NaCl concentration produced an improvement of extraction efficiency before 30% w/v of the content of NaCl because of the salting-out effect. However, with further increasing of the content of NaCl, the extraction efficiency decreased. The

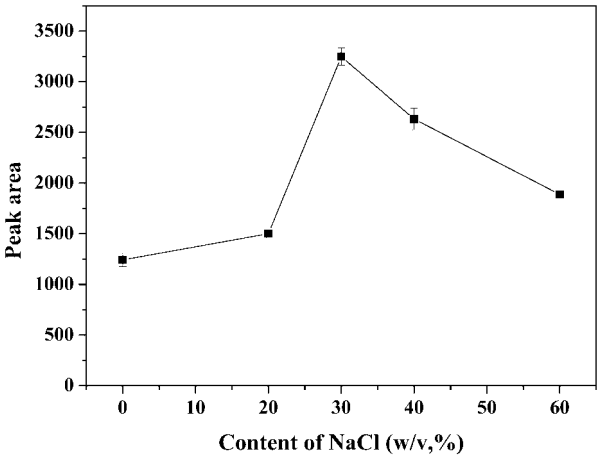

Figure 4. Effect of content of NaCl on peak area of colchicine extracted by the IL-SDME-CE. Other IL-SDME conditions are the same as in Fig. 2, except the content of NaCl. The error bars represent the standard deviation.

probable explanation is that the addition of salt may increase the viscosity of sample solution so that the diffusion of colchicine to IL drop is suppressed. For this reason, a NaCl concentration of 30% w/v was chosen as optimal condition for ionic strength of the sample solution.

3.2.5 Effect of aqueous phase sample volume

Aqueous phase sample volume is also an important factor in conventional extraction process. To study the effect of aqueous phase volume on extraction efficiency, the volumes of 400, 500, 600, 700, and 800 μL were examined, respectively. As shown in Fig. 5, the best extraction efficiency was achieved when aqueous phase volume was 600 μL. It is because there is sufficient target analyte when volume is 600 μL. However, with further increasing of the

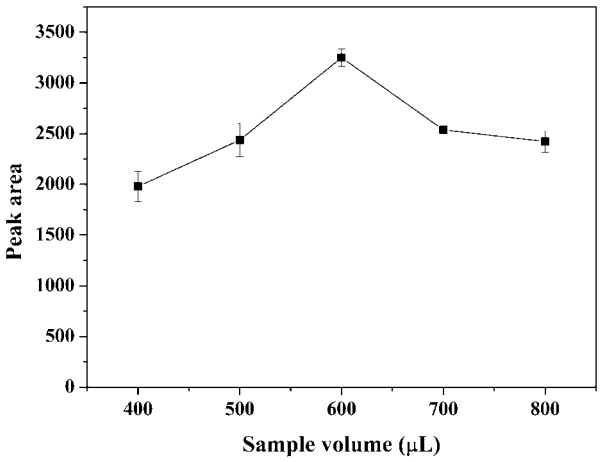

Figure 5. Effect of sample volume on peak area of colchicine extracted by the IL-SDME-CE. Other IL-SDME conditions are the same as in Fig. 2, except the sample volume. The error bars represent the standard deviation.

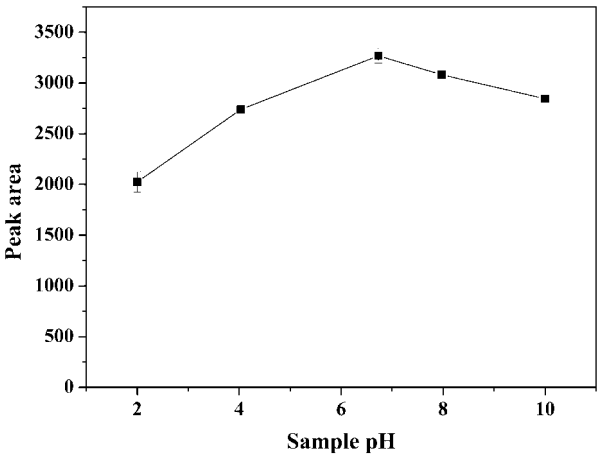

Figure 6. Effect of sample pH on peak area of colchicine extracted by the IL-SDME-CE. Other IL-SDME conditions are the same as in Fig. 2, except the sample pH. The error bars represent the standard deviation.

aqueous phase volume, there is a process that sample solution may dissolve IL single drop, which has adverse influence on extraction efficiency. Therefore, the aqueous phase sample volume of subsequent experiments was set at 600 μL.

3.2.6 Effect of sample pH

In an extraction method, sample pH is always adjusted to enhance the extraction efficiency. The effect of sample pH was investigated across the range of 2.00–10.00. As shown in Fig. 6, the biggest peak area of the colchicine was obtained at nearly neutral condition which was the sample aqueous solution without adjusting pH. The peak area under acid or alkaline condition was decreased. Based on the molecular structure of the colchicine, there is an ester group which can be hydrolyzed and become amido under acid or alkaline condition. In the acid condition, the amido may combine with H^+, which has adverse effect on extraction. Meantime, the H^+ could effect with PF_6^-, which could speed up the dissolution of IL. The peak area of the colchicine after hydrolysis showed a loss. Thus, the sample pH was set at neutral condition in the subsequent experiments.

3.3 Method validation

To investigate the method performance of the proposed IL-SDME-CE method for determining target colchicine in aqueous sample, a series of experiments were designed. A lot of parameters were investigated under optimal conditions, including linear range, linearity, LOD, and limit of quantitation (LOQ). Typical electrophoretograms are shown in Fig. 7. The coefficient of variation (CV) of peak area for colchicine was 6.4% at 5 μg/mL ($n = 5$). The linear range studied for colchicine was 1–100 μg/mL. The correlation coefficient (R^2) for colchicine was 0.9994 which was obtained by averaging the peak area for each concentration. The LOD, calculated as the ratio of single-to-noise (S/N) of 3, was 0.25 μg/mL. The LOQ was 0.83 μg/mL ($S/N = 10$). The EF for colchicine is 41 which was the ratio of concentrations after IL-SDME and initial sample solution at 50 μg/mL with the relative standard deviation of 2.8% ($n = 3$).

In Table 1, analytical methods about detection of colchicine are summarized. As summarized in Table 1, LOD of colchicine on HPLC or HPLC-MS is lower than this study's. These result from the low detection sensitivity of CE inherently. However, compared with detection on microchip capillary electrophoresis (MCE), LOD of this study is decreased by about tenfold of that. Besides, compared with [11], LODs of MEKC and SPE-CE are about 24-fold and 2-fold of this study's, respectively. Thus, it can be seen that this IL-SDME-CE method has lower LOD than the other methods on CE, namely, the better detection sensitivity. Besides, compared with other methods, this IL-SDME-CE is enrichment, separation, and detection all in one set with satisfactory extraction time, sample requirement, and analysis time. The LOD of the IL-SDME-CE is in the low μg/mL range, which is propitious to monitor the toxicity of colchicine in food products as well as analysis of colchicine in other real sample on CE. The proposed online IL-SDME-CE method for extraction and determination of colchicine is simple, convenient, and low-cost.

3.4 Application to real sample

In order to investigate the applicability of this method for the determination of colchicine in real sample, Lanzhou lily sample was used for model. The aqueous extract of Lanzhou

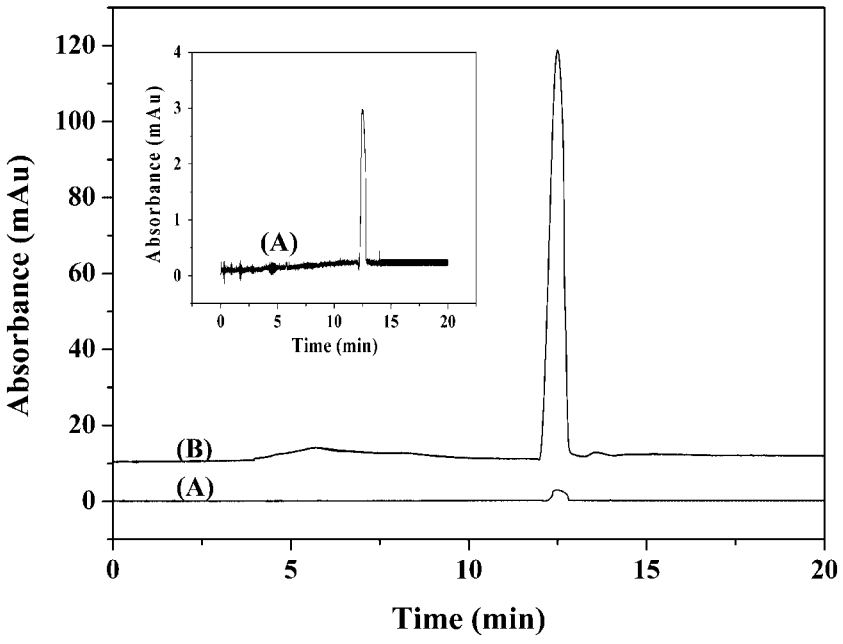

Figure 7. Typical electrophoretograms of cochicine: (A) normal injection of cochicine (50 μg/mL) without extraction; (B) distilled water with spiked colchicine (50 μg/mL) enriched by IL-SDME. The inset figure shows the enlarged view of normal injection.

Table 1. Analytical parameters comparison of analytical methods on detection of colchicine[a)]

Detection methods	Extraction time (min)	Sample volume (mL)	EF	Analyzing time (min)	Linear range	LOD	Reference
TLC-densitometry	5	–	–	–	Nonlinearity	2.1 μg/mL	[6]
HPTLC	–	–	–	–	5–35 μg/mL	1 μg/mL	[37]
HPLC	190	–	–	12.7	1.0–250 μg/mL	0.05 μg/mL	[38]
HPLC	5	0.2	–	13	2–900 ng/mL	0.5 ng/mL	[39]
SPE-HPLC	–	0.5	–	26	1–200 ng/mL	4 ng/mL	[40]
LC-ESI-MS-MS	10	3	–	8	0.0005–1 mg/L	0.0001 mg/L	[1]
LC-MS-MS	10	0.1	–	2.5	0.05–10 ng/mL	35 pg/mL	[9]
MCE	–	3	6	35	5.0–500 mg/L	2.4 mg/L	[10]
SPE-CE	–	2	14	15	10–50 μM	1.1 μM	[11]
MEKC	–	2	–	2.5	200–1000 μM	15 μM	[11]
This study	10	0.6	41	14	1–100 μg/mL	0.25 μg/mL	

a) HPTLC, high-performance thin-layer chromatography; MCE: microchip capillary electrophoresis.

lily was directly used for IL-SDME-CE, but no colchicine was detected. The aqueous extract sample was spiked with colchicine at two concentrations, 5 and 50 μg/mL, both obtaining good results. The average recovery results were 102.4 and 98.8%, respectively.

4 Concluding remarks

A simple, green, and convenient method was established for determining colchicine in real sample with sufficient reproducibility, precision, and sensitivity on CE. The method provided short analytical time, low LOD, and wide linear range. The proposed method can purify the real sample, which is suitable for monitoring the toxicity of colchicine in food products. For real sample determination, the results of IL-SDME-CE showed successful application for separation and preconcentration of low-concentration colchicine in spiked aqueous extract of Lanzhou lily sample, showing that IL-SDME can be a promising way for sample preparation in colchicine analysis. Additionally, the present study widens the applicability of novel IL-SDME-CE method further which contributes to the development of online miniaturization sample preparation technology in CE.

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The authors have declared no conflict of interest.

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