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Matrix solid-phase dispersion extraction coupled with HPLC-diode array detection method for the analysis of sesquiterpene lactones in root of *Saussurea lappa* C.B.Clarke

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ABSTRACT

We developed a reliable and effective method to determine costunolide and dehydrocostuslactone in the root of *Saussurea lappa* C. B.Clarke using matrix solid-phase dispersion (MSPD) extraction, HPLC separation and diode array detection (DAD). Several extraction parameters for the MSPD were optimized. Florisil was chosen as dispersing adsorbent with methanol as elution solvent. The ratio of Florisil to sample was selected to be 4:1 and no additional clean-up steps were needed. Linearities ($r > 0.9995$) were determined to be in the range of 22.5–360.0 μg/mL for costunolide and 25.0–400.0 μg/mL for dehydrocostuslactone. Intra- and inter-day precisions were also determined with a relative standard deviation (RSD) less than 3.2%. The limits of detection were found to be 0.122 μg/mL for costunolide and 0.135 μg/mL for dehydrocostuslactone. The recoveries were in the range of 92.5–99.8% with relative standard deviations ranged from 1.2% to 3.5%. The proposed MSPD method required shorter time and lower solvent volume than maceration–ultrasonic and Soxhlet extraction methods.

1. Introduction

Traditional Chinese medicine (TCM), which applies the natural medicinal plants under the guidance of the theory of TCM science, plays an indispensable role in the prevention and treatment of diseases in China. As a result of its high pharmacological activity, low side effect and rare complication, TCM is becoming more and more popular throughout the world [1]. The root of *Saussurea lappa* C.B.Clarke (family Compositae), a TCM material, has been recorded as "MuXiang" in the Chinese Pharmacopoeia for a long time [2]. It is mainly used for treatments of many digestive system diseases, including gastric and abdominal pain, loss of appetite, indigestion, diarrhea, anorexia, nausea and vomiting [3]. In addition, this herb can also be used to treat asthma and cough [4,5], coronary heart disease [5], acute pancreatitis, acute cholecystitis and hepatitis [6,7]. The extensive literature investigations of *Saussurea lappa* C.B.Clarke revealed that sesquiterpene lactones costunolide and dehydrocostuslactone are the major active compounds [8–13]. These compounds have been found to involve in many pharmacological activities, such as anti-ulcer [14], anti-cancer [15–18], hepatoprotective [10] and cytotoxic properties [13]. Furthermore, they also have been found to exhibit activities of antiangiogenic [19], anti-inflammatory [20], antimicrobial and fungicidal [21,22] and immunomodulatory [23].

For analyses of costunolide and dehydrocostuslactone in plants or in biological matrices, many methods have been established using HPLC–UV [24–26], TLC–densitometry [27], ^{13}C-NMR spectroscope detection [28], high-speed counter-current chromatography [29] and HPLC–MS [30]. Different sample preparation methods have also been applied to extract costunolide and dehydrocostuslactone from the roots of *Saussurea lappa* C.B.Clarke. The classical methods to extract the target compounds include solvent extraction and maceration extraction. In many cases, extraction methods and following clean-up steps are vital for the success of analyzing the target compounds, because of the extreme complexity of the medicinal plant matrix. However, the classical extraction methods are usually time consuming, labor intensive, complicated and require large amounts of solvent and sample. Therefore, the development of a simple and effective extraction method is of great interest in the recent years. As a result, many new extraction techniques have been developed, including ultrasound assisted extraction, microwave assisted extraction, pressurized-liquid extraction, supercritical fluid extraction, solid-phase micro extraction and liquid-phase micro extraction [31].

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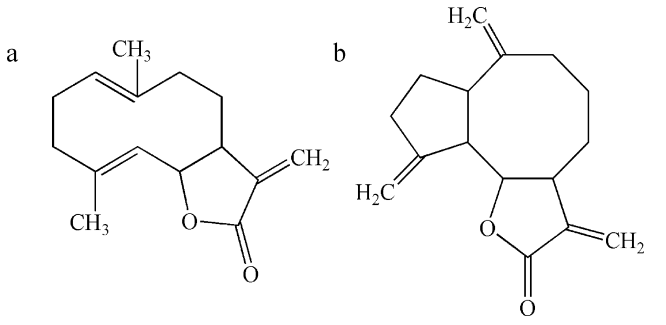

Fig. 1. The chemical structures of (a) costunolide and (b) dehydrocostuslactone.

As an attractive alternative, MSPD has recently been introduced for sample preparation of complex matrices. This technique, first developed in 1989 by Barker et al. [32], has been successfully applied for the isolation of different types of drugs, micro-contaminants and naturally occurring compounds from a wide variety of sample matrices [33]. The successful application of MSPD to solve many difficult analytical problems have evidenced its great potentials to reduce analyst time, increase sample throughput and shorten turn-around time, reduce solvent use and the attendant expense of solvent purchase and disposal, as well as provide analytical results that are equal to or better than classical or official methods [34]. However, there are only limited amount of published papers using MSPD as sample preparation method to extract constituents in medicinal plants [35–40]. No literature has been reported to apply MSPD as a sample preparation method for the analyses of sesquiterpene lactone components in medicinal plants.

In this study, MSPD as extraction method followed by HPLC separation and diode array detection was first applied to extract and determine costunolide and dehydrocostuslactone in the roots of *Saussurea lappa* C.B.Clarke. The effects of several extraction parameters, including dispersing sorbent, elution solvent, volume of the elution solvent and the ratio of dispersing sorbent to sample, were examined and optimized. The results obtained in real samples with the optimized MSPD procedure were evaluated and compared with the classical Soxhlet and official maceration–ultrasonic extraction methods.

2. Experimental

2.1. Chemicals and reagents

Reference standards of costunolide (purity ≥98%) and dehydrocostuslactone (purity ≥98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of the two compounds are shown in Fig. 1. Three different roots of *Saussurea lappa* C.B.Clarke (named as samples 1–3) cultivated in different area were bought from local drugstore. These materials were identified and authenticated by Professor Lina Guo (Pharmacognosy Department of Qiqihar Medical University). The roots were crushed and passed through a 100 mesh sieve. The obtained samples were stored in a desiccator at room temperature.

HPLC-grade methanol was received from Dikma Technology Inc. (Richmond, USA). Analytical-grade methanol, acetone, ethyl acetate, chloroform and light petroleum were purchased from Concord Technology Co. Inc. (Tianjin, China). The ultrapure water used was prepared via a Purelab plus water purification system (PALL, USA).

Dispersing Sorbents tested for MSPD including Florisil (particle size 75–150 μm) was obtained from Dikma Technology Inc. (Richmond, USA). C18-bonded silica (particle size 40–63 μm) was obtained from SiliCycle (Quebec, Canada). Silica gel (particle size 48–75 μm) was obtained from Qingdao Haiyang Chemical Subsidiary Factory (Qingdao, China). Multi-walled carbon nanotubes (MWCNT, 3–5 nm i.d., >233 $m^2 g^{-1}$), hydroxyl modified multi-walled carbon nanotubes (OH-MWCNT, 3–5 nm i.d., >233 $m^2 g^{-1}$, OH-content (weight): 3.7%) and carboxyl modified multi-walled carbon nanotubes (COOH-MWCNT, 3–5 nm i.d., >233 $m^2 g^{-1}$, COOH-content (weight): 2.56%) were all obtained from Chengdu Organic Chemical Co. Ltd. (Chengdu, China).

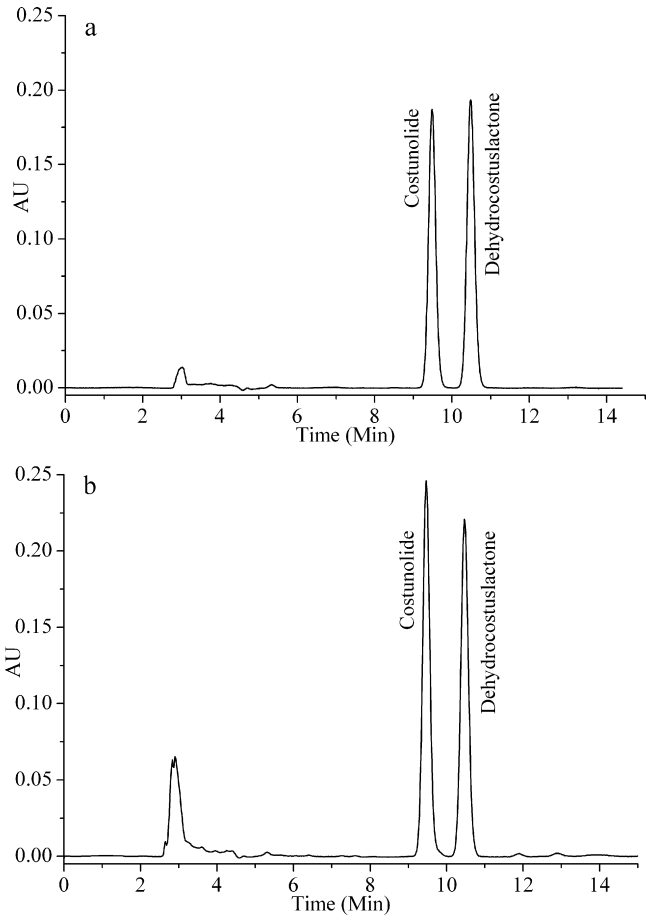

Fig. 2. Representative HPLC chromatograms of (a) standard mixture solutions (90 μg/mL for costunolide and 100.6 μg/mL for dehydrocostuslactone). (b) *Saussurea lappa* C.B.Clarke sample extracted using MSPD.

2.2. HPLC and chromatographic conditions

Chromatographic analyses were performed with an Beckman GOLD series high performance liquid chromatograph equipped with a dual solvent pump (model 125 solvent module), a DAD detector (model 168 detector) and a Rheodyne injection valve (model 7725i) with 20 μL sample loop. The chromatographic separation of the compounds was achieved with an Agilent TC-C18 column (5 μm, 250 mm × 4.60 mm) at room temperature. The mobile phase was made up of methanol and water (70:30, v/v) at a flow rate of 1 ml/min. The injection volume was 20 μL and the 225 nm was selected as the detection wavelength. The chromatographic signals were monitored and integrated by use of Beckman Gold software. The representative chromatograms are shown in Fig. 2.

2.3. Preparation of standards

The two sesquiterpene lactones were accurately weighed and then dissolved with methanol to prepare stock solutions. The costunolide and dehydrocostuslactone standard stock solutions were stored at 4 °C and brought to room temperature before use. Working solutions were prepared by diluting the stock solutions with the mobile phase.

2.4. Sample preparation

2.4.1. MSPD extraction

An aliquot of 0.3 g of the previously milled sample and 1.2 g of Florisil were placed in an agate mortar and blended together using an agate pestle to obtain a complete disruption and dispersion of the sample on the Florisil sorbent. Once completely dispersed, the homogeneous mixture was transferred into the cartridge containing absorbent cotton at the bottom. A second layer of absorbent cotton was placed on the top of the mixture by careful compression with a syringe plunger. Elution was carried out with 10 mL of methanol by gravity flow. And then the purpose analytes were eluted out and collected in a 10 mL of volumetric flask. An aliquot of 1 mL eluate was further diluted to 5 mL with mobile phase. This solution was filtered through a 0.45 μm PTFE membrane and used as the sample solution.

2.4.2. Maceration–ultrasonic extraction

This extraction method was used as an official method in the Pharmacopoeia of China [2].

Typically, 0.3 g of the sample was placed in a 100 mL conical flask, followed by the addition of 50 mL methanol as the extraction solvent. After macerated for 24 h at room temperature, the conical flask was immersed in the water bath of an ultrasonic cleaner (KQ-5200 DE Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China), the extraction process was conducted for 30 min of ultrasonication at room temperature and the output power was set at 200 W. Then, the extract was diluted to 50 mL with methanol. The resulting extract was centrifuged and the clear solution filtered through a 0.45 μm PTFE membrane before HPLC analysis.

2.4.3. Soxhlet extraction

0.3 g of sample and 90 mL of methanol were put into a Soxhlet distilling flask. The mixture was heated and refluxed for 20 h. The extract was transferred into a 100 mL of volumetric flask and diluted to the mark with methanol. After filtered through a 0.45 μm PTFE membrane, the resulting solution was used as the sample solution for HPLC analysis.

2.5. Stability

In order to demonstrate the stability of both standard and sample solutions, stability of standard solutions and sample extracts were measured. No significant degradation for stock standard solutions were checked and found to be stable at least 1 month at 4 °C. Concentration differences for working standard solutions were less than 0.3% in 7 days at 4 °C. Concentration differences for sample extracts maintained at room temperature for 24 h were less than 1%. All of them were sufficient to complete the whole analytical process.

3. Results and discussion

3.1. Optimization of the MSPD procedure

MSPD is one of the most promising techniques for the simultaneous disruption of samples and extraction of analytes. In this work, the MSPD extraction was examined as a preparation technique for the isolation of costunolide and dehydrocostuslactone from the roots of *Saussurea lappa* C.B.Clarke. Different conditions that affect MSPD extraction such as dispersing sorbent, elution solvent, volume of the elution solvent and the ratio of adsorbent to sample were studied. A further clean-up procedure was also checked.

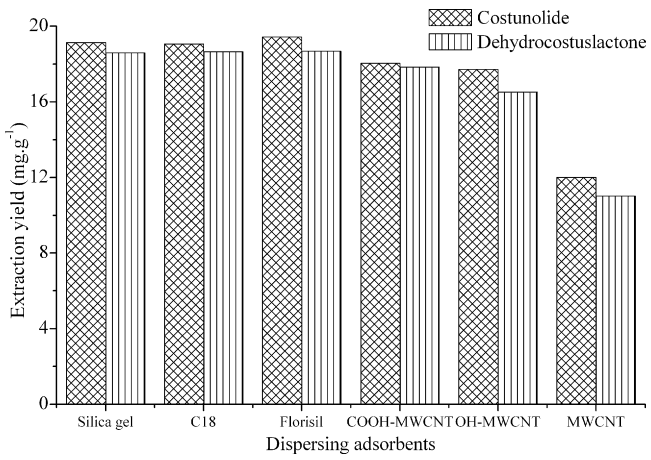

Fig. 3. The effect of the dispersing adsorbents on extraction yields of costunolide and dehydrocostuslactone.

3.1.1. Effect of dispersing sorbent

In the MSPD procedure, the dispersing sorbent is used as not only an adsorption separation material but also a blending solid support to disrupt and disperse the sample. The influence of the dispersing sorbent was initially studied with methanol as elution solvent. Several dispersing adsorbents including silica gel, Florisil, C18, MWCNT, OH-MWCNT and COOH-MWCNT were examined in order to find the most suitable dispersing adsorbent.

Fig. 3 depicts the extraction yields of the sesquiterpene lactones from the roots of *Saussurea lappa* C.B.Clarke using different dispersing adsorbents. It was found that the yields of the sesquiterpene lactones obtained with three kinds of MWCNT were all lower than those obtained with the other three sorbents. However, the extracts obtained by using three kinds of MWCNT were cleaner than those obtained by using C18, silica gel and Florisil as dispersing sorbent. OH-MWCNT and COOH-MWCNT have higher extraction yields than MWCNT due to stronger interaction between target molecule and COOH-MWCNT or OH-MWCNT including hydrogen bonding and electrostatic interaction. Among all the dispersing adsorbents that examined, Florisil obtained highest yield for sesquiterpene lactones with relatively low cost. As a result, Florisil was selected as the dispersing sorbent.

3.1.2. Effect of ratio of dispersing sorbent to sample

The four different ratios of Florisil to sample mass: 1:1, 2:1, 3:1, and 4:1, were tested. The experimental results in Fig. 4 shows that the mass ratio has no significant effect on the extraction yields of sesquiterpene lactones. But when the mass ratio is 4:1, the extraction yields of costunolide and dehydrocostuslactone are all slightly higher than other mass ratios. Thus this ratio was selected in this study.

3.1.3. Effect of elution solvent and volume of the elution solvent

Polarity of the elution solvent is another important parameter in the MSPD extraction. We investigated five popular solvents used in the MSPD with different polarities, including methanol, acetone, ethyl acetate, chloroform and light petroleum. Methanol was selected as elution solvent for the further work, because it shows better result in Fig. 5 than any other solvent.

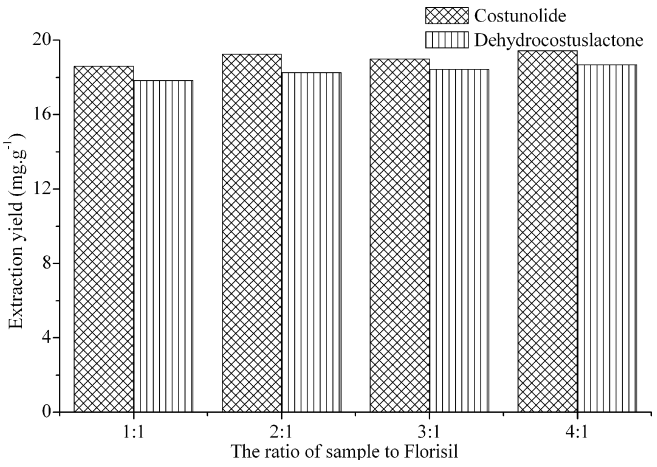

Fig. 4. The effect of the ratio of adsorbent to sample on the extraction yields.

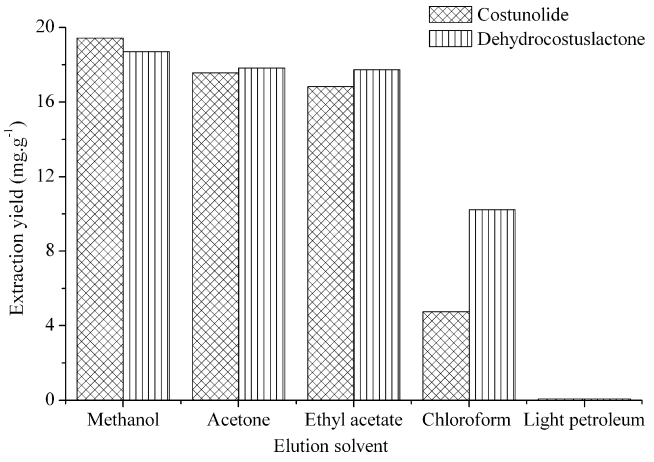

Fig. 5. The effect of elution solvents on the extraction yields.

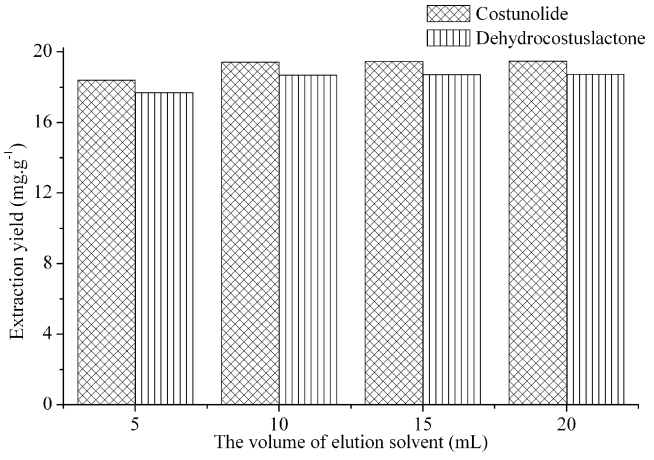

Fig. 6. The effect of volume of elution solvent on extraction yields.

Additionally, effect of elution solvent volume on extraction yields of target compounds was also investigated. As shown in Fig. 6, the extraction yields of costunolide and dehydrocostuslactone slightly increased when the volume of methanol was from 5 to 10 mL. But when the volume of methanol increased from 10 to 20 mL, the extraction yields did not have obvious change. Finally, the volume of methanol was established at 10 mL to ensure efficient extraction while reduce consumption of solvent.

3.1.4. Effect of clean-up sorbents

A further clean-up procedure was tested using silica gel and C18, which were packed at the bottom of the MSPD cartridge as the clean-up sorbents for cleaning the extract. There was no significant difference in HPLC chromatograms obtained with and without clean-up sorbents. However, this clean-up procedure led to lower extraction yields of sesquiterpene lactones than the extraction procedure without any clean-up. So clean-up sorbents were not used in this work.

3.2. Validation of the HPLC method

3.2.1. Linearity

Linearities of the calibration standards were tested at six concentration levels in the concentration range of 22.5–360.0 μg/mL for costunolide and 25.0–400.0 μg/mL for dehydrocostuslactone. Good linearity between the peak area and concentration of the analyte was obtained throughout the concentration range, and the regression equations were $y = 0.4293x - 0.0712$ for costunolide and $y = 0.4332x - 0.7238$ for dehydrocostuslactone with the correlation coefficients of 0.9995 and 0.9996, respectively. Where y is the peak area, x is the concentration in μg/mL.

3.2.2. Limit of detection and limit of quantification

The standard stock solutions were further diluted with methanol to provide a series of solutions with the appropriate concentrations. The limit of detection (LOD) and quantification (LOQ) for each compound were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio were about 3 for LOD and 10 for LOQ, respectively. The LODs for costunolide and dehydrocostuslactone were 0.122 and 0.135 μg/mL. The LOQs for costunolide and dehydrocostuslactone were 0.405 and 0.450 μg/mL.

3.2.3. Precision

The precision of the proposed method was assessed by study of repeatability and intermediate precision. Repeatability (intra-day) of the assay method was evaluated by six replicates of MSPD extraction sample solution in one day and the relative standard deviation of six values was calculated to determine intra-day precision. Intermediate precision (inter-day) at the same sample solution was determined on three successive days. The percentage RSD values for the precision study were 1.5, 1.4% (intra-day precision) and 2.5, 3.2% (inter-day precision) for costunolide and dehydrocostuslactone, respectively. These confirmed good precision of the proposed method.

3.2.4. Recovery

To assess the method's accuracy, extractions were carried out at two fortification levels, and each test performed in triplicate. The *Saussurea lappa* C.B.Clarke samples were fortified with the standard stock solutions and followed by an air-drying for 24 h at ambient temperature. This procedure must be careful to avoid the loss of target compounds. Unspiked "blank" samples were previously analyzed to determine the presence of costunolide and dehydrocostuslactone. As shown in Table 1, the recoveries were in a range of 92.5–99.8% for costunolide and dehydrocostuslactone with RSDs ranged from 1.2% to 3.5% in all fortification levels. Excellent recoveries were made in all fortification levels. It was confirmed from the results that the proposed method is highly reliable and consistent.

3.3. Comparison of MSPD, maceration–ultrasonic and Soxhlet extraction procedure

In order to evaluate the performance of optimized MSPD, the real samples were submitted to the optimized MSPD,

Table 1
Recovery studies of costunolide and dehydrocostuslactone from samples with known concentration.

Compounds	Added amount (mg/g)	Original amount (mg/g)	Found amount (mg/g)	Recovery (%)	RSD (%, $n = 3$)
Costunolide	9.9	19.43	29.24	99.1	3.5
	9.9	19.43	28.77	94.3	
	9.9	19.43	28.61	92.7	
	18.0	19.43	36.08	92.5	2.7
	18.0	19.43	36.74	96.2	
	18.0	19.43	36.96	97.4	
Dehydrocostuslactone	9.1	18.68	27.76	99.8	3.4
	9.1	18.68	27.16	93.2	
	9.1	18.68	27.43	96.2	
	17.3	18.68	35.10	95.2	1.2
	17.3	18.68	35.33	96.6	
	17.3	18.68	35.56	97.6	

Table 2
Comparison of MSPD, Maceration–ultrasonic and Soxhlet extraction.

	MSPD	Maceration–ultrasonic	Soxhlet
Extraction yield of costunolide (mean ± SD[a], mg/g)	19.43 ± 0.51	18.55 ± 0.44	17.44 ± 0.49
Extraction yield of dehydrocostuslactone (mean ± SD, mg/g)	18.68 ± 0.37	17.65 ± 0.49	16.78 ± 0.54
Sample (g)	0.3	0.3	0.3
Solvent (mL)	10	50	100
Time (h)	0.5	25	20.5

[a] Standard deviation ($n = 3$).

Table 3
Results of HPLC analysis of samples.

Samples	Costunolide		Dehydrocostuslactone	
	Extraction yield (mg/g)	RSD (%, $n = 3$)	Extraction yield (mg/g)	RSD (%, $n = 3$)
No.1	19.43	2.6	18.68	2.0
No.2	17.64	3.1	16.94	1.6
No.3	14.02	3.0	13.38	2.9

maceration–ultrasonic and Soxhlet extraction procedures. Then the extracts were analyzed by HPLC–DAD. It can be seen from these data (Table 2) that the extraction yields of costunolide and dehydrocostuslactone for the MSPD procedure were higher than maceration–ultrasonic and Soxhlet extraction. Moreover, the MSPD procedure required shorter time, lower solvent volume and involved fewer extra equipment in the determination of costunolide and dehydrocostuslactone in the roots of *Saussurea lappa* C.B.Clarke than maceration–ultrasonic and Soxhlet extraction procedures. And also the MSPD procedure did not require heating during the extraction avoided the possible loss and degradation of the costunolide and dehydrocostuslactone. Took into account the extraction yields and consumption of time and solvent, MSPD extraction should be a relatively better method.

3.4. Application in real sample analyses

This proposed method that combines MSPD and HPLC–DAD was applied to analyze three real samples cultivated in different area. The analytical results are summarized in Table 3. In all three samples, the contents of costunolide and dehydrocostuslactone are in the range of 14.02–19.43 mg/g and 13.38–18.68 mg/g, respectively. Due to the possible differences in cultivated areas, growth conditions and picking periods, the differences in costunolide and dehydrocostuslactone contents in these samples are quite evident. However, all three samples meet the content requirement about "MuXiang" in Pharmacopoeia of China.

4. Conclusion

In this study, a MSPD extraction method was proposed in combination with HPLC–DAD for an efficient and reliable determination of costunolide and dehydrocostuslactone in the roots of *Saussurea lappa* C.B.Clarke. Compared with maceration–ultrasonic and Soxhlet extraction techniques, the MSPD extraction requires smaller amount of samples (0.3 g), lower consumption amount of organic solvents (10 mL) and shorter extraction time (0.5 h). In addition, subsequent clean-up steps are not required. The proposed method has been successfully validated in terms of linearity, precision, reproducibility and recovery, proving that this new method can be used as an advantageous alternative procedure for routine analysis of target compounds.

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