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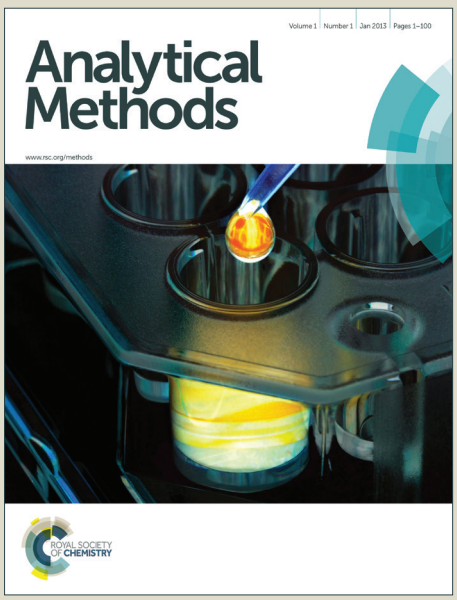

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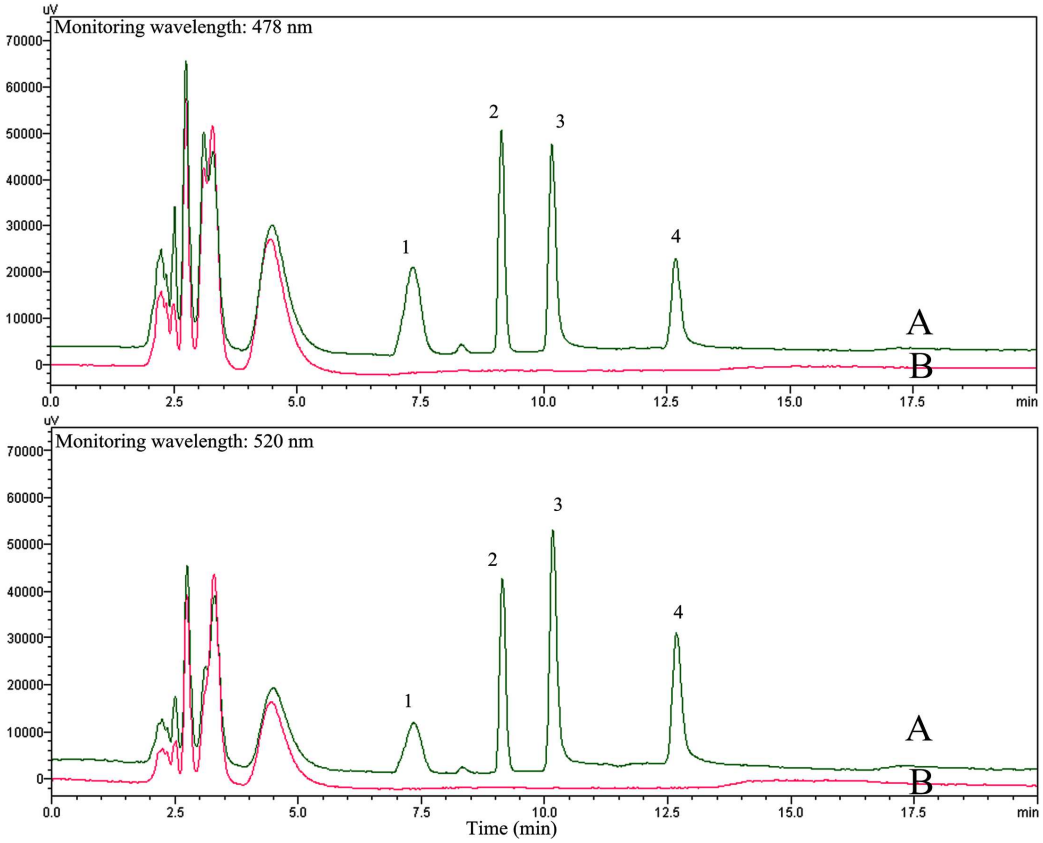

A simple, rapid and effective extraction method based on MSPD and UFLC has been developed and validated for the determination of illegal dyes from the roots of Salvia miltiorrhiza Bunge.

Determination of illegal dyes in Salvia miltiorrhiza Bunge by matrix solid phase dispersion and ultrafast liquid chromatography

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Abstract

A simple, rapid and effective extraction method based on matrix solid phase dispersion (MSPD) and ultrafast liquid chromatographic (UFLC) was developed and validated for the simultaneous cleaning-up and quantitative extraction of illegal dyes (Sudan I~IV) from the roots of Salvia miltiorrhiza Bunge. The experiment parameters, such as dispersing sorbent, the ratio of sorbent/sample, washing solvent and elution solvent were evaluated to find the optimal MSPD conditions. The optimal conditions were 0.5 g of Salvia miltiorrhiza Bunge, 1.0 g of silica gel as dispersing sorbent, a volume of 10 mL of water as the washing solvent and 4 mL of acetonitrile-methnaol (9:1, v/v) as the elution solvent. Under these conditions good linearity for all the Sudan dyes was ranged from 0.10 μg g^{-1} to 10 μg g^{-1} ($r^2 \geq 0.9992$). The recoveries at three spiked levels (0.1, 1.0, 5.0 μg g^{-1}) were between 80.6% and 96.1% with relative deviations (RSDs) ranging from 2.3% to 8.6%. The limits of detection were ranged between 0.013 and 0.024 μg g^{-1} which were twenty times lower than the values required by European regulations. This method has potential to be applied for the determination of illegal dyes in complicated traditional Chinese herb materials.

1. Introduction

In recent years, food and drugs safety is an increasingly important health issue. Adding illegal synthetic drugs and illegal dyes in health food and traditional Chinese medicines becomes one of the major concerns. There are always some unscrupulous traders who take chance to add illegal additives in health food and traditional Chinese medicines to deceive consumers for windfall profits, since the market itself has no moral criteria to guarantee the security of goods. Food and drugs safety not only concern the healthy life of people, but also the sustainable development of human society. Therefore, to ensure the complete absence of illegal additives in food and drugs, we need legal tools as well as effective and sensitive analytical techniques to validate them.

Sudan dyes (Sudan I~IV) are a group of synthetic colorants which are widely used in many chemical industries and daily products due to their low price, bright color and fastness. They belong to the family of lipophilic azo dyes which usually have one or more azo groups (-N=N-) and are traditionally used in printing inks, waxes, candles, oils, plastics, textiles, cleaners, etc.[1] However, azo dyes have been questioned constantly, many compounds of this family have been shown to be carcinogenic in animal experiments.[2] Among which, Sudan dyes appear to be genotoxic carcinogen and mutagen to human, and they have been categorized as class 3 carcinogen by International Agency for Research on Cancer (IARC).[3,4] Sudan dyes used in food products are strictly banned worldwide according to both the Food Standards Agency (FSA) and the European Union (EU). EU has established the detection limits for Sudan dyes in foodstuffs at 0.5-1 mg kg^{-1}.[5] Because of their low cost and the enhancement of products appearance, Sudan dyes are still illegally utilized as additives in a variety of food products by many unscrupulous traders. Up to date there are very few researches or regulations about the illegal additives in traditional Chinese medicines. Owing to the low levels of Sudan dyes in traditional Chinese medicine samples and the complexity of the sample matrices, it is very difficult to determine these compounds directly. Therefore it is crucial to develop an analytical approach

with a simple and fast preparation procedure for the isolation and enrichment of Sudan dyes.

Salvia miltiorrhiza Bunge is an ancient Chinese herb which belongs to the family of Labiatae. The morphology of Salvia miltiorrhiza Bunge are shown in Fig. 1. The dried root of Salvia miltiorrhiza Bunge has been commonly used in traditional Chinese medicine used for the treatment of various diseases, especially cardiovascular diseases including coronary heart disease, hypertension, and chronic heart failure.[6] Owing to its bright red skin, it is commonly called "Danshen" in Chinese. According to modern chemical and pharmacological investigations, the dried roots of Salvia miltiorrhiza Bunge have various chemical constituents, such as phenolic acids, tanshinones, flavonoids, triterpenes, sterols, pigments and polysaccharides.[7,8] Among which, the hydrophilic phenolic acids and lipophilic tanshinones are the major bioactive components of this herb.[9,10] The content of phenolic acids and the lipophilic tanshinones is also considered as the quality criterion of Salvia miltiorrhiza Bunge by the Chinese Pharmacopoeia.[11] Lu et al. (2013) investigated the relationship between the root skin color and the content of major bioactive components and confirmed that the best-quality Salvia miltiorrhiza Bunge were those with a brownish-red skin color.[12] There are always some unscrupulous traders who take chance to add illegal dyes to improve the appearance of dried roots of Salvia miltiorrhiza Bunge for windfall profits. To ensure there are no illegal dyes in Salvia miltiorrhiza Bunge, analytical methods used in laboratories require not only a novel sample pretreatment technique with high enrichment factor and desirable selectivity, but also advanced analytical instruments to guarantee an accurate and sensitive analytical result.

Until now, a lot of analytical methods have been developed for the detection of Sudans dyes, such as immunoassay method,[13,14] liquid chromatography,[15] liquid chromatography-mass spectrometry (LC-MS),[16,17] voltammetry,[18] electrophoresis,[19] chemiluminescence analysis.[20] Various sample preparation techniques have been developed as well, including solid-phase extraction (SPE),[21-22] cloud point extraction (CPE),[23] ultrasonic-assisted extraction (UAE),[24] ionic liquids extraction[25] and stir bars

microextraction.[26] They are usually multi-step procedures, typically based on exhaustive extraction from the matrix into organic solvents or separated by solid phase extraction and the subsequent removal of co-extracted material by several clean-up steps prior to instrumental analysis. Although each method has its advantages, there are still many deficiencies in all these extraction methods, such as rigorous conditions, complicated operations, time consuming, organic solvent wasting and lack of selectivity. In addition to that, the sample matrices of Salvia miltiorrhiza Bunge are too complex for the common methods. Therefore, a simple, accurate and practicable method for the identification and quantification of Sudan dyes in traditional Chinese medicines should be developed.

Matrix solid phase dispersion (MSPD) is a simple and cheap sample preparation procedure which enables the simultaneous extraction and clean-up of analytes from various solid and semi-solid materials.[27,28] The MSPD procedure involves dispersion of the sample over a suitable dispersing sorbent, subsequent preliminary purification and elution of the analytes with a relatively small volume of solvent.[29,30] Due to the variety of combinations of sorbents and elution solvents, this technique shows high flexibility and selectivity. MSPD can also be used as an auxiliary technique to classical extraction methods with significant reduction in solvent consumption and requires no particularly expensive instruments.[31] MSPD has been mainly applied to the analysis of organic environmental pollutants and of several additives in food and biological matrices,[32-34] but to our knowledge it has not been used for analyzing the illegal dyes in traditional Chinese medicine matrices.

The aim of the current study was to develop and validate a practical, easy and economical method based on MSPD and ultrafast liquid chromatographic (UFLC), while realize the cleaning-up and quantitative extraction of illegal dyes (Sudan I~IV) from the roots of Salvia miltiorrhiza Bunge. The effects of several parameters, such as type of dispersing sorbent, the ratio of sorbent/sample, volume of washing solvent and elution solvent were investigated to find the optimal MSPD conditions. This method has potential to be applied for the rapid detection of Sudan dyes in complicated

traditional Chinese herb materials at LOD low enough for quantitative determination under European legislation.

2. Experimental

2.1 Chemicals and reagents

HPLC-grade solvents used were acetonitrile (ACN) and methanol (MeOH), obtained from Fisher Scientific Company (USA). All other reagents used were of analytical grade or better and were purchased from Beijing Chemical Works (Beijing, China). Deionized water, with a resistivity of 18.2 MΩ cm, was obtained using a Milli-Q water purification system (Millipore Co., USA). Solid phase materials used for MSPD were Celite® 545 (diatomaceous earth) with median pore size of 17 μm from J&K Scientific Ltd. (Beijing, China), florisil (150-250 μm), alumina N and silica gel (40-63 μm) supplied by Westingarea Corporation (Shanghai, China). All sorbents were activated at 600 °C for 4 h, dried at 100 °C for 2 h and then allowed to cool down in a desiccator before use. Empty polypropylene solid-phase extraction (SPE) syringes (15 mL capacity) and polyethylene frits (20 micron porosity) were purchased from Bonna-Agela Technologies Inc. (Tianjin, China).

The solid standards of Sudan I (CAS no. 842-07-9; purity 97%), Sudan II (CAS no. 3118-97-6; purity 90%), Sudan III (CAS no. 85-86-9; purity 96%), and Sudan IV (CAS no. 85-83-6; purity 92%) were obtained from National Institutes for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of the Sudan dye are shown in Fig. 2. Stock solution of each standard was prepared in acetonitrile at a concentration level of 0.1 mg mL^{-1} and stored at 4 °C in darkness. Mixed working standard solutions were prepared fresh daily by diluting the stock solutions with acetonitrile.

2.2 Equipments

A UFLC-UV system (Shimadzu, Japan) equipped with two LC-20AD pumps, a

SIL-20A automatic sample injector, a CTO-20A column oven and a SPD-20A UV–vis detector was used for the analysis of Sudan dyes. Relevant data acquisition and processing were performed with LC-solution software (Shimadzu, Japan). A high-speed universal disintegrator (FW-100) was purchased from Taisite Instrument Co., Ltd. (Tianjin, China). Rational speed of the high-speed universal disintegrator is 24,000 r min^{-1} and motor power is 460 W.

2.3 Sample preparations

Five kinds of dried roots of Salvia miltiorrhiza Bunge cultivated in different areas were bought from local drugstores. After being cleaned, the samples were dried in the oven at 50 °C for 24h before being crushed by the high-speed universal disintegrator. After being disintegrated, the samples were passed through an 80 mesh sieve. The sample powder was stored at room temperature in a desiccator until use. Recovery experiments were performed by spiking the blank Salvia miltiorrhiza Bunge sample with a proper volume of standard working solution in a glass mortar. After being well homogenized, the sample was equilibrated in the dark at room temperature overnight before being analyzed.

2.4 MSPD procedure

MSPD extractions were carried out considering different type of dispersing sorbents, washing solvents, elution solvents and ratio of sorbent/sample. Under the optimal operation conditions, a sample amount of 0.50 g was poured into a glass mortar with 1.0 g of silica gel sorbent. The mixture was blended with a glass pestle for 2-3 minutes to obtain a homogeneous material for the MSPD column. Thereafter, the blend was transferred to a 15-mL polypropylene SPE syringe with a polyethylene frit on the bottom. The second frit was placed over the dispersed sample and then the mixture was slightly compressed with a syringe plunger. The column was washed by gravity flow with 10 ml of water. After this solvent had flowed through the column, 1

mL of ACN-MeOH (9:1,v/v) (about the dead volume of the column) was eluted while a positive pressure was applied with a syringe plunger to remove any remaining solvent and the eluate was rejected. The analytes were eluted by adding 4 mL of ACN-MeOH (9:1,v/v) to the column. The eluent was collected in a 10 mL conic tube and was evaporated to dryness under a stream of nitrogen at 70 °C, re-dissolved with 200 μL ACN. Finally, the resulting solution was filtered with 0.22 μm filters and placed in a 300 μL-insert, which was inserted in a 1.5 mL-vial for UFLC-UV analysis.

2.5 UFLC-UV analysis

UFLC analyses were performed using a Shimadzu (Kyoto, Japan) UFLC system. A VP-ODS column (150 mm × 4.6 mm, with 4.6 μm particle size) was employed for the separation and determination of Sudan dyes. The mobile phase consisted of acetonitrile (A) and water (B). The gradient profile was carried out starting from 0% to 60% B in 5 min, then to 0% B in 1 min and remained the same until the end of the run. Separations were performed at a flow rate of 1.0 mL min^{-1} and each separation was stopped after 20 min. The monitoring wavelength was 478 nm for sudan I and 520 nm for sudan II~IV. The temperature of column was controlled at 30 °C. Injection volume was 20 μL.

3. Results and discussion

3.1 Optimization of MSPD method

In this study, the extraction and purification method proposed is based on the MSPD procedure, which is an auxiliary technique of extraction in complex matrices. MSPD involves dispersion of the sample over a suitable dispersing sorbent, subsequent preliminary purification and elution of the analytes with a relatively small volume of solvent. In order to optimize the MSPD method, several experimental parameters affecting the extraction and purification efficiency were studied, including type of

dispersing sorbent, type and volume of washing solvent, type and volume of elution solvent, ratio of sorbent/sample. A pre-optimization for the experimental parameters was carried out with blank controlled samples spiked at 5 μg g^{-1} and based on the preselected optimum parameters, the effects of experimental parameters were investigated again. When one parameter changed, the other parameters were fixed at the preselected optimal values. All the experiments were performed in triplicate.

3.1.1 Type and amount of dispersing sorbent. The first step in the method development was the choice of a suitable dispersing sorbent. In order to achieve extracts with the highest recovery and the lowest amount of matrix interferences, four dispersants, such as florisil, Celite, alumina-N and silica gel were investigated. According to the MSPD extraction procedure mentioned in the "Experimental" section, preliminary experiments were carried out by blending the sample (500 mg) with one of the selected dispersants at the ratio of 1/2. The efficiency of the solid support was studied using 3 mL of ACN as the elution solvent. An initial clean-up step with 10 mL of water was necessary to remove the interfering compounds such as phenolic acid compounds and other water-soluble compounds from the sample prior to analytes elution. According to the results, Celite and silica gel showed more potential for extracting Sudan dyes than the other two dispersing sorbents mentioned above. Since Sudan dyes are strongly hydrophobic compounds, just as expected, for their effective isolation from the sample matrix the dispersion of the sample with polar dispersing sorbent is very attractive.

Although there was no significant difference in terms of recovery studies for silica gel and Celite, silica gel was considered the optimum choice for the subsequent MSPD experiments. The main reason is that it produced cleaner extracts as evidenced by the visual inspection of the extracts and by the number and intensity of peaks in the chromatographic profiles measured with the UV detector. Furthermore, when Celite was used as the dispersing sorbent, elution and washing of the sample required much more time due to the low permeability of the bed and small particle size of the sorbent.

Therefore, silica gel was selected as the dispersing sorbent in the following experiments.

In the MSPD extraction procedure, the chosen dispersing sorbent is not only used as adsorbent separation material but also as a solid support during the blending process to disrupt and disperse the sample. A suitable amount of dispersing sorbent could increase the interface area between the sorbent and analytes, and facilitate analytes transfer into sorbent by allowing complete adsorption of the sample components. In this work, the ratio of sample/sorbent, from 1:1 to 1:4, was evaluated in the MSPD procedure. A volume of 10 mL of water was used in the initial clean-up step and 3 mL of ACN was used as the elution solvent. Best recoveries were obtained when the ratio of sample/sorbent was 1/2. Then this value was established in subsequent experiments.

3.1.2 Type and volume of washing solvent. Salvia miltiorrhiza Bunge can be considered a difficult matrix due to its chemical composition. During the extraction procedure high numbers of compound are co-extracted. For this reason, after loading the sample, it is important to apply a washing step which ensures the reduction of the matrix interference and prevents an excess of interfering compounds from polluting analytical column. During the period of pre-optimization, a preliminary wash of the MSPD column with a non polar solvent n-hexane was carried out to eliminate non polar and less polar interfering compounds. Subsequently, water was used to eliminate phenolic acid compounds and other water-soluble compounds from the sample. Unexpectedly, after extraction, there was a small amount of n-hexane residue in the eluent which can clog the piping of the UFLC-UV system and cause the liquid chromatographic column pressure rise. Furthermore, the presence of small amount of n-hexane residue in the SPE column decreased the contact area of the matrix with other solvent and in return, the accuracy and precision of the MSPD method should decrease accordingly. Thus, the non polar solvent n-hexane was abandoned in this work and water combine with a series of water-soluble organic solvents was

investigated in subsequent experiments.

To find a suitable washing solvent, a volume of 10 mL of different solvents included, water, MeOH-water (2:8, 1:1, v/v) and EtOH-water (2:8, 1:1, v/v) was investigated as the washing solvent respectively. By adding a small amount of alcohol to the water, the matrix interference was dramatically reduced as evidenced by the visual inspection of the extracts and by the number and intensity of peaks in the chromatographic profiles, whereas the recoveries were much lower than those obtained with pure water. Therefore, water was selected as the washing solvent in the following experiments. For the purpose of minimum volume of washing solution able to efficiently rinse the interferences, different volumes of water (5-12 mL) were investigated. According to the results, the recoveries of Sudan dyes increased with the increase of washing volume from 5 to 10 mL and then retained constant even further increasing the volume to 12 mL. Therefore, the volume of 10 mL was selected as the optimum washing volume for the subsequent experiments.

3.1.3 Type and volume of elution solvent. The solvents selected for elution are strictly connected to the matrix nature and analyte polarity. To find a suitable elution solvent, a volume of 3 mL of different solvents included, ACN, MeOH, ethanol (EtOH), ACN-water (9:1, v/v), ACN-EtOH (9:1, v/v) and ACN-MeOH (9:1, v/v), was tested as the elution solvent respectively. According to the results, best recoveries were obtained using ACN-MeOH (9:1, v/v). The average recoveries of Sudan dyes were improved by adding a small amount of MeOH to the ACN, whereas the recoveries using ACN-water were much lower than those obtained with pure ACN. The elution solvent in this work is not only used as a general mobile phase in the SPE column but also used to selectively dissolve/extract target compounds from samples. Thus, the lower recoveries obtained with ACN-water as elution solvent can be attributed to the lower solubility of Sudan dyes in the water-containing solvent. Based on the obtained results, ACN-MeOH (9:1, v/v) was selected as the elution solvent for all further experiments.

To optimise the elution volume, different volumes of ACN-MeOH (9:1, v/v) (2-6 mL) were invested. The recoveries of Sudan dyes increased with the increase of elution solvent from 2 to 4 mL, and then slightly decreased. The reason may be that the higher the volume of elution solvent used, the higher the amount of interferences eluted out simultaneously. In view of almost all the interferences in the resulting solution present signal enhancement or suppression, it is necessary to select a suitable volume of elution solvent that allows the elution of the largest number of target component but the least number of co-extractants. Therefore, 4 mL was used as the optimum volume of elution solvent.

3.2 Validation of the method

Under the optimal experimental conditions, a series of experiments were performed for investigating linearity, precision, recovery, limits of detection (LODs) and quantification (LOQs).

3.2.1 Linearity. Calibration curves were constructed by plotting the areas of the chromatographic peaks versus the increasing spiked concentrations in the range of 0.10-10 μg g^{-1}. All the experiments were performed in triplicate. As shown in Table 1, good linearity was obtained throughout the concentration range for the four Sudan dyes with correlation coefficients (r^2) ≥ 0.9992.

3.2.2 LODs and LOQs. LODs and LOQs were considered as the lowest concentration of a certain analyte that can be detected and quantified for a given confident level, respectively. They were related to the value of statistical fluctuations of the zero signal and the magnitude of analytic signal. The method LODs and LOQs ranging from 0.013 to 0.024 μg g^{-1} and from 0.044 to 0.078 μg g^{-1} indicated in Table 1 are determined at the lowest concentration which yielding a signal-to-noise (S/N)

ratio of 3 and 10, respectively. All the LODs of analytes were at low ng g^{-1} level which were twenty times lower than the values required by European regulations. The results shown in Table 1 indicate that LODs, LOQs and linear ranges are appropriate to the goal of the proposed method.

3.2.3 Accuracy and precision. Accuracy and precision of the MSPD-UFLC method were assessed at three spiking levels in terms of intra-day repeatability (n=6) and inter-day reproducibility (three alternative days). Accuracy was evaluated by the recoveries of the analytes at three spiking levels (0.1, 1.0, and 5.0 μg g^{-1}). Precision was evaluated by measuring the intra-day and inter-day RSDs. The results obtained are shown in Table 2. The intra- and inter-day precision are in the range of 2.3-6.3% and 3.1-8.6%, respectively. The recoveries at three spiked levels (0.1, 1.0, 5.0 μg g^{-1}) were between 80.6% and 96.1%. These results show that the accuracy and precision of the present method were satisfactory. The typical UFLC chromatograms obtained from the spiked and blank Salvia miltiorrhiza Bunge sample are shown in Fig. 3.

3.3 Real sample analysis.

In order to evaluate the applicability of the proposed method, a series of experiments were conducted to analyze the concentration of Sudan dyes in five real Salvia miltiorrhiza Bunge samples from different growing areas. Three replicate determinations were carried out for each sample. As listed in Table 3, no Sudan dyes were found in the tested samples. To study the effect of sample matrix, recovery experiments were carried out by spiking two different concentrations (0.1 and 1.0 μg g^{-1}) of Sudan dyes into Salvia miltiorrhiza Bunge samples. The average recoveries for all the analytes were in the range of 81.1-100.3% with RSD < 8.4%, which indicated that the MSPD-UFLC method was reliable and could be used for the detection of trace levels of Sudan dyes in other complicated plant materials.

4. Conclusion

In this study, a simple, rapid and effective MSPD-UFLC method for the simultaneous determination of Sudan dyes from the roots of Salvia miltiorrhiza Bunge has been developed. The experiment parameters affecting MSPD process were optimized, such as dispersing sorbent, the ratio of sorbent/sample, washing solvent and elution solvent. The analytical performances are satisfactory. LODs obtained by the proposed method were in the range of 0.013-0.024 μg g^{-1}, twenty times lower than the values established by European regulations. The recoveries at three spiked levels (0.1, 1.0, 5.0 μg g^{-1}) were between 80.6% and 96.1% with relative deviations (RSDs) ranging from 2.3% to 8.6%. This method should be applied for the determination of illegal dyes in other complicated plant materials by varying the extraction conditions.

Acknowledgements

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Table 1 Analytical parameters of the proposed method.

Analyte	Linear range ($\mu g\ g^{-1}$)	Calibration equations	Correlation coefficient (r^2)	LOD ($\mu g\ g^{-1}$)	LOQ ($\mu g\ g^{-1}$)
Sudan I	0.10~10	A=91690.8×c-3081.5	0.9998	0.013	0.044
Sudan II	0.10~10	A=80556.7×c-2748.3	0.9998	0.014	0.048
Sudan III	0.10~10	A=128516.7×c-14068.8	0.9998	0.024	0.078
Sudan IV	0.10~10	A=97329.3×c-13735.2	0.9992	0.024	0.078

425

Table 2 The intra- and inter-day precision and recoveries (n=6).

Analyte	Added ($\mu g\ g^{-1}$)	Intra-day precision		Inter-day precision	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Sudan I	0.1	87.3	5.4	88.3	5.7
	1.0	93.2	5.0	90.0	4.3
	5.0	94.8	4.3	91.3	5.6
Sudan II	0.1	90.3	4.1	96.1	3.5
	1.0	92.3	4.0	90.3	3.1
	5.0	89.4	6.3	86.0	5.4
Sudan III	0.1	94.3	6.2	88.1	5.7
	1.0	85.4	3.7	87.5	4.2
	5.0	87.3	3.6	85.4	8.6
Sudan IV	0.1	88.3	8.5	80.6	5.1
	1.0	86.3	2.3	88.9	6.7
	5.0	91.1	6.2	89.1	5.5

Table 3 Real sample analysis (mean±SD, n=3)

Analyte	Added ($\mu g\ g^{-1}$)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Sudan I	0	-	-	-	-	-
	0.1	88.2±5.5	90.2±2.2	91.6±1.1	84.2±7.5	84.6±6.5
	1.0	97.2±2.3	95.7±3.0	95.6±2.4	89.8±8.4	90.2±4.0
Sudan II	0	-	-	-	-	-
	0.1	90.3±3.2	90.7±2.8	100.3±3.2	81.3±7.7	95.6±3.2
	1.0	92.7±3.0	90.5±1.8	95.4±2.7	87.7±7.0	88.7±3.0
Sudan III	0	-	-	-	-	-
	0.1	95.3±4.2	90.5±3.4	95.2±4.0	89.9±2.4	90.3±4.2
	1.0	85.6±4.2	89.8±2.2	90.6±2.2	83.3±8.2	86.6±3.2
Sudan IV	0	-	-	-	-	-
	0.1	89.3±4.8	85.7±7.3	93.4±1.5	86.9±6.0	91.3±1.9
	1.0	88.3±5.3	86.7±4.3	98.6±0.8	89.0±6.5	89.3±2.2

430

Figure captions

Fig. 1. Morphology of Salvia miltiorrhiza Bunge.(A) Portion above ground. (B) Roots for traditional Chinese medicine use.

Fig. 2. Chemical structures of he four Sudan dyes.

Fig. 3. UFLC chromatograms obtained from Salvia miltiorrhiza Bunge sample spiked at 5μg g^{-1} level of Sudan Ⅰ ~ Ⅳ subjected to the complete MSPD method (A), and non-spiked or blank sample (B) at monitoring wavelength 478 and 520nm.

1, Ⅳ; 2, Sudan Ⅱ; 3, Sudan Ⅲ; 4, Sudan Ⅳ.

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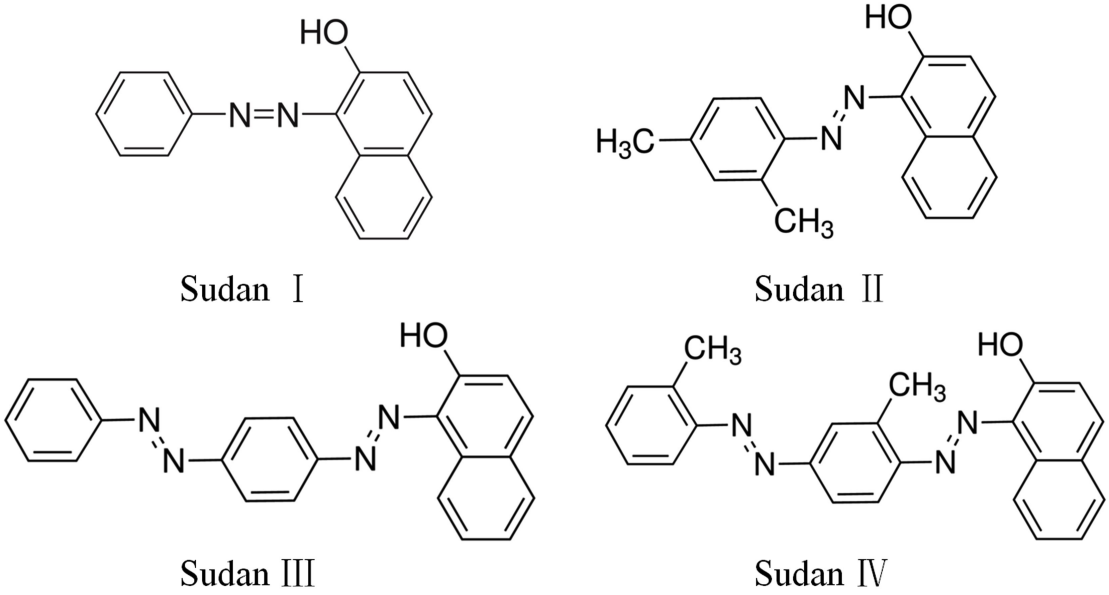

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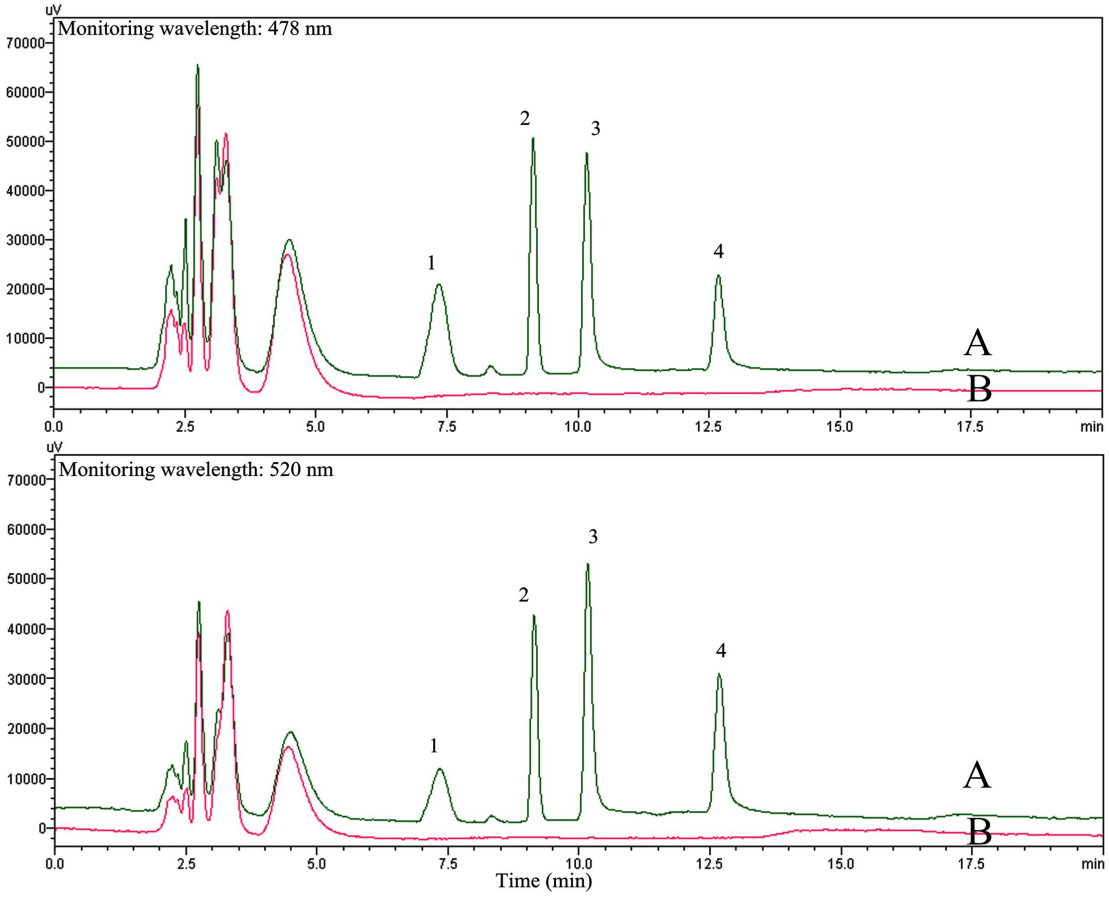

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