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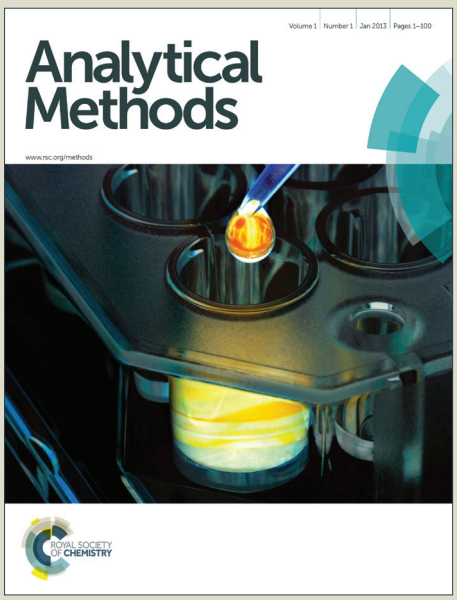

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Organic solvent-free matrix solid phase dispersion (MSPD) for determination of synthetic colorants in chilli powder by high-performance liquid chromatography (HPLC-UV)

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ABSTRACT

A green and simple organic solvent-free matrix solid phase dispersion (MSPD) was developed and applied to the extraction of four synthetic colorants (amaranth, sunset yellow, allura red and erythrosine) in chilli samples. High-performance liquid chromatography (HPLC) coupled with an ultraviolet (UV) detector was used for the separation and determination of the analytes. The chromatographic separation was performed on a Phenomenex Luna C18 (2) 100A column in a gradient using methanol/water, as the mobile phase at a flow rate of 0.8 mL·min^{-1} and 35 □ of temperature. Factors such as type of dispersant, ratio of sample to dispersant, type, volume, pH value and ionic strength of elution solvent were investigated. Under the optimum experimental conditions, the linearities for determining the analytes were in the range of 30-2000 ng·g^{-1} for amaranth and erythrosine, 20-2000 ng·g^{-1} for sunset yellow and allura erythrosine. Limits of detection were ranged between 8.6 and 13.5 ng·g^{-1}. No organic solvents were used in the extraction procedure, and 4 mL water directly eluted the analytes. Thus, the method avoided environmental pollution of organic solvents and removed multi-step procedures in the sample pretreatment and has potential to be applied using a simple instrument presented in most analytical laboratories.

Key words[1]

High performance liquid chromatography (HPLC); matrix solid phase dispersion (MSPD); colorants; chilli powder

1. Introduction

Food colorants, which not only make foods more attractive for the consumers but also replace their natural color that can be lost during the preparation process or storage, have been widely used as additives in the food industry [1-4]. Food colorants can be classified into natural colorants and synthetic colorants based on their sources. Natural colorants unstable and easily undergo variations during the food processing and storage [5-9]. Synthetic colorants, as important class of food additives, usually have lower production cost, bright and clear colours and greater stability against light, heat and acids than natural colorants [5, 10-13]. Therefore, many kinds of synthetic colorants are still widely used in food industry to compensate the lacks of natural colors. However, the negative influence of synthetic colorants on human organism was confirmed by some researchers. Some synthetic colorants especially those containing azo functional groups and aromatic rings can lead to allergic and asthmatic reactions, DNA damage, hyperativity, respiratory problem, thyroid tumours and chromosomal damage when taken orally or absorbed by skin[6, 14

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-18]. To ensure the safety of food supply, content of synthetic colorants in foods must be strictly controlled and reliable methods for the determination of synthetic colorants are required. There are a lot of analytical techniques to carry out the identification and determination of synthetic colorants in foods, such as electrochemical [19], capillary electrophoresis (CE) [20, 21], chemiluminescence [22], UV-visible spectrophotometry [23, 24]，gas chromatography (GC) [25], ultra-performance liquid chromatography (UPLC) [26], high-performance liquid chromatography [11]. Compared with other techniques, liquid chromatography coupled with mass spectrometric (MS) detection [14, 26, 27, 28]，ultraviolet (UV) detection [29, 30] and diode-array detection (DAD) [11, 31, 32, 33, 34] is the most common technique for separating and determining synthetic colorants.

Whichever determination method is selected, an effective sample pretreatment and enrichment process are necessary due to the complexity of sample matrices and low levels of analytes. In recent years, several new pretreatment methods including molecularly imprinted polymers for solid phase extraction (MISPE) [30, 35, 36], dispersive liquid-liquid microextraction (DLLME) [15, 30], liquid-phase microextraction (LPME) [37], have been reported for the pretreatment of food samples. These are usually multi-step procedures including the elimination of proteins and lipids, and the subsequent removal of co-extracted material by several clean-up steps before instrumental analysis. Therefore, these methods are more time-consuming and tedious. For example, in the MISPE procedure MISPE stationary phase has to be synthesized beforehand. In addition, most of these methods could not be directly applied for semi-solid and solid samples which must be pretreated into solution prior to adapt those extraction procedures. Therefore, simple and efficient method is required to ensure the safety of food supply.

Matrix solid phase dispersion (MSPD), a process for simultaneous homogenization, extraction and clean-up of samples, was first developed in the late 1980s by Barker et al. for the extraction of semi-solid, solid and highly viscous samples. Compared with classical liquid-liquid or liquid-solid phase extractions of solid and semi-solid samples, MSPD can eliminate most of the interference particularly complex biological samples [38, 39]. The basic procedure of MSPD involves dispersion of the sample matrix with an appropriate dispersant, loading the mixture of sample and sorbent into small column and subsequent washing and elution of the analytes with suitable solvents [32, 40, 41]. More recently, many solid supports, such as under-ivatized silicates (silica gel, sand, etc.), organic (graphitic fibers) or inorganic (Florisil, alumina, etc.) solids, can be used as dispersants in the MSPD process [38]. MSPD has been applied to the analysis of several additives in foods and satisfactory results were observed [30, 42, 43].

To the best of our knowledge, there is no report on the simultaneous extraction of amranth allura red, sunset yellow and erythrosine in chilli samples by MSPD method. So, this work aims to develop a simple and green MSPD-HPLC-UV method to allow the extraction and determination of four colorants (amranth, allura red, sunset yellow and erythrosine) in chilli samples. The chemical structures of the analytes are shown in Fig.1. Following the rapid development of analytical techniques, the movement toward less organic solvent consumption and faster extraction time is a trend in analytical method [44], so in this MSPD process no organic solvents was used to avoid environmental pollution of organic solvents in the sample pretreatment. The effects of various experimental parameters were studied and optimized. The proposed method was applied to the analysis of chilli samples.

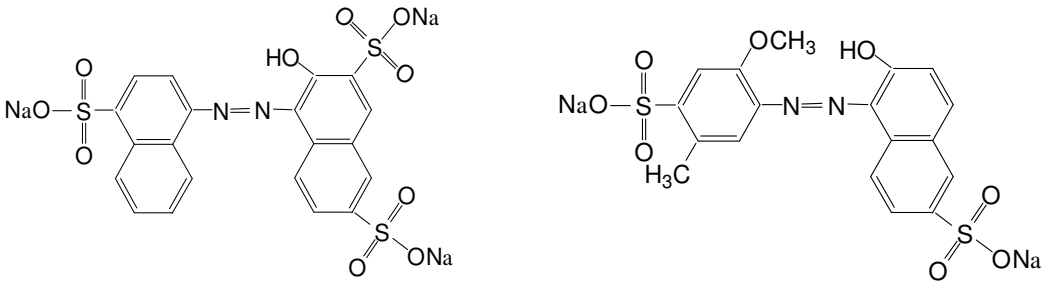
2. Experimental

2.1. Chemicals and materials

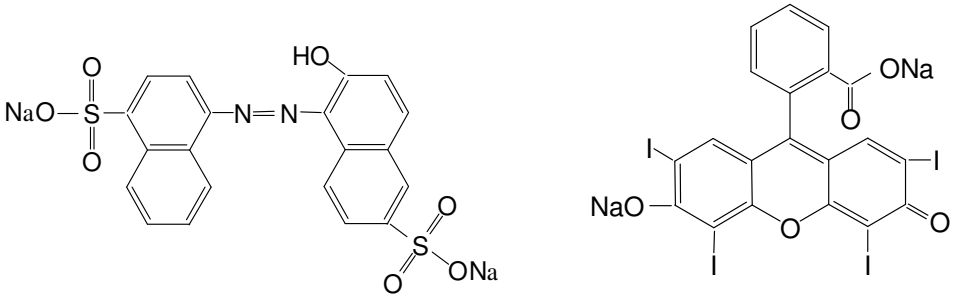
Amranth, allura red, sunset yellow and erythrosine were obtained from the National Research Center for Certified Reference Materials (Beijing, China). Chromatographic grade methanol and hydrochloric acid was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Analytical grade ammonium acetate was obtained from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China). C18 (100-200 mesh), Silica gel (200-300 mesh), diatomite (200-300 mesh), Florisil (60-100 mesh), acidic alumina (100-200 mesh), neutral alumina (200-300 mesh) and basic alumina (200-300 mesh) were obtained from Chinese Medical and Biological Products Institute (Beijing, China). Pure water was obtained with a Milli-Q water purification system (Millipore Co., USA).

2.2. Instruments

Chromatographic separation of target analytes was performed on a Phenomenex Luna C18 (2) 100A column (150mm × 4.6 mm I.D., 5 μm). A Shimadzu LC-20AT liquid chromatograph equipped with a DGU-20A5 degasser, a quaternary pump (L2130), a UV-Vis Detector system (L2420) and a CTO-10AS column oven (Shimadzu Corporation, Japan) was used. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 μL. A high speed centrifugation (TGL-16C, Shanghai, China) was employed to accelerate the phase separation process. A DELTA-320 acidity meter (Mettler-Toledo Instruments Co., Ltd, Shanghai, China) was used for pH measurement. A (RE-52CS-1, Shanghai, China) rotatory evaporator was used for the concentration.



Amranth **Allura Red**



Sunset Yellow **Erythrosine**

Fig.1 Chemical structure of analytes.

2.3. Preparation of standards and sample

The standard stock solution for each colorant at the concentration level of 500 $\mu g \cdot mL^{-1}$ was

prepared by dissolving the analyte in methanol, and stored at 4 ºC in the dark. The working solutions were prepared by mixing and dilution the standard stock solution with methanol. Three chilli powder samples which were purchased from different local supermarkets (Chongqing, China) were blended for 2 min and were dried thoroughly in the air blast oven at 60 ºC for 8 h. The samples then were passed through a 60 mesh stainless steel sieve and stored in a refrigerator at 4 ºC. Recovery experiments were performed by spiking chilli powder samples with the desired amount of each colorant.

2.4. MSPD procedure

A representative portion of chilli powder (0.1 g) was placed into a glass mortar and 0.4 g of Florisil was added. The chilli powder was then gently blended into the Florisil material with a glass pestle, until a homogeneous mixture was obtained. Afterwards，the homogeneous mixture was packed into a 10 ml syringe barrel with a layer of absorbent cotton at the bottom, a second layer of absorbent cotton was covered on the head of the sample mixture. The packing material was slightly compressed using a piston to a final height of 7 mm. Then 4 mL water was added to the column and the sample was allowed to elute dropwise by applying a slight vacuum. The eluate was collected in a 5 mL conic tube and concentrated to dryness using a rotary vacuum evaporator (60 ºC) after centrifugation for 5 min at 8000 rpm. The residue was dissolved in 0.2 ml water. The resulting analytical solution was filtered with 0.22 μm PTFE filter membrane before HPLC analysis.

2.5. Chromatographic analyses

Solvent A (0.2% ammonium acetate) and solvent B (methanol) were combined in a gradient as follows: 0-5 min,15-50% B; 5-10 min, 50-85% B; 10-15 min, 85-85%B; 15-20 min, 85-15%B. The flow rate of mobile phase was 0.8 $mL \cdot min^{-1}$, the column temperature was kept at 35 ºC and the UV detector was set at a wavelength of 520 nm for amranth, allura red, sunset yellow and erythrosine. The injection volume of the sample solution was 20 μL.

3. Results and discussion

3.1. Optimization of MSPD extraction procedure

Extraction and clean-up conditions had to be carefully selected to achieve the highest recovery for the colorants while eliminating most of the interfering matrix components. The most important factors in MSPD procedure including type of dispersant, ratio of sample to dispersant, type and volume of elution solvent, pH value and ionic strength of the elution solvent, were investigated. For the MSPD parameter optimisation, chilli powder was spiked with a concentration level of 500 $ng \cdot g^{-1}$ of amranth, allura red, sunset yellow and erythrosine.

3.1.1. Type of dispersant and ratio of sample to dispersant

One of the major steps in the development of the MSPD method was to select the sorbent for sample dispersion. In this study, few sorbents with different physical and chemical properties including silica gel, diatomite, florisil, basic alumina and neutral alumina were examined. Both the polarity of the dispersants and the extraction solvent determine the efficiency of the extraction and the cleanness of the final extracts. Strong retention of the analytes was observed, only small amount of analytes were eluated in the tests using basic alumina and neutral alumina as dispersants. The reason may be that the analytes were adsorbed onto the dispersants. When diatomite, Florisil and silica gel were used as the dispersants，recoveries of analytes obtained with Florisil were higher than those obtained with diatomite and silica gel. In addition, strong background absorption was observed in the chromatographic analyses when diatomite and silica

gel was used as the dispersants. The results reported in Fig.2. show that only Florisil proved the highest analyte recoveries among those tested. Therefore, it was selected as dispersants acting as dispersing agents of the sample for all further experiments. The effect of mass ratios of sample/Florisil, including 1:2, 1:3, 1:4, 1:5, 1:6 1:7, were tested. As indicated in Fig.2, best recoveries were obtained when the mass ratio was 1/4, enhancing the Florisil ratio gave no improvement. So the mass ratio was selected for all the experiments.

3.1.2. Type and volume of elution solvent

In trace analysis, residue levels are close to the limit of sensitivity of the instrument, even trace matrix components can interfere with determination, so that a suitable elution solvent is critical in MSPD method [45]. Theoretically, an appropriate solvent or solvent mixture should allow the elution of analytes, free of matrix components [46]. Because the four colorants in this study are highly water-soluble polar compounds, in order to obtain satisfactory recoveries, water, acetonitrile, methanol and ethanol were assayed as elution solvents. Interference chromatograms were observed when acetonitrile, methanol and ethanol were used as elution solvents. What is more, the elution of acetonitrile, methanol and ethanol result in low recoveries of the analytes. The reason may be that some nonpolar compounds eluted by organic solvents interfered chromatogram peaks. Clean and free of interference chromatogram in the tests using water as elution solvent was observed. Because the four colorants are highly water-soluble polar compounds, target analytes were easily eluted from the column but non polar and low polar compounds still retained on MSPD column when the water was used as elution solvent. Thus, water was selected as elution solvent. To optimise the eluent volume, several trials were conducted using different volumes of water (2, 3, 4, 5 and 6 mL). The obtained results depicted in Fig.3 showed that 4 mL water allowed the hingher recoveries than other volumes. Therefore, 4 mL water was chosen as the elution solvent in further experiments.

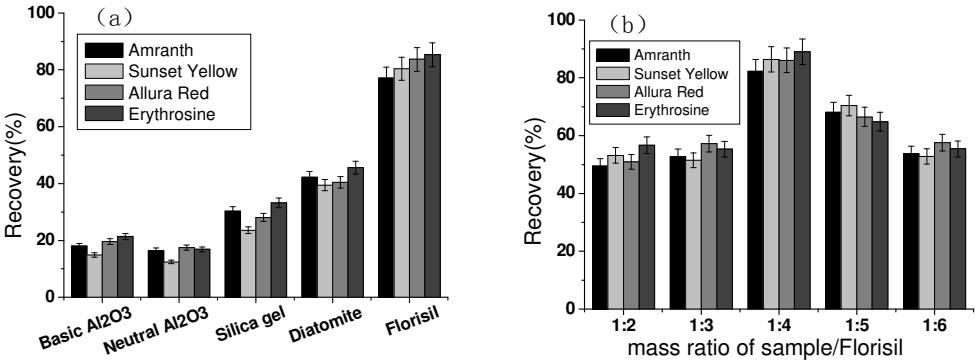


Fig.2 Influence of the sorbent (a) and mass ratios of sample/Florisil (b) on MSPD procedure. Spike level: 500 ng·g^{-1}, eluant: water.

3.1.3. Effect of pH value of elution solvent

The pH value of the solution determines the present state of analytes [15]. Therefore, the pH value plays an important role in the separation of the colorants. The effect of different pH value (2.0, 4.0, 5.0, 5.8, 8.0, 10.0 and 12.0, hydrochloric acid was used to adjust pH) of the elution solvent on the recoveries was examined. As can be seen from Fig.3, the recoveries of the target analytes increase significantly especially the erythrosine with the increase of pH values ranging from 2.0 to 5.8,

then decrease slightly with the increase of the pH values ranging from 5.8 to 12.0. The reason may be that the instability of erythrosine at low pH contributed to the low recoveries of erythrosine. Based on the experimental results, the pure water (pH=5.8) was the best compromise.

3.1.4. Effect of clean-up sorbent

Trying to reduce the matrix effect and to simplify the chromatograms, preliminary wash of the MSPD column with 3 mL n-hexane was carried out to eliminate non polar and low polar compounds prior to analytes elution. Then 4 mL water was used as the elution of the analytes. However, compared with absence of n-hexane wash, it was seen that the use of n-hexane leads to no significant difference in HPLC chromatograms. The reason may be that the poor solubility in water of non polar and low polar compounds attributed to the similar clean chromatograms. So n-hexane was not used in this work.

3.1.5. Effect of ionic strength

To evaluate the effect of ionic strength on the extraction of target analytes, different amount of NaCl was added in the elution solvent. The recoveries of the target compounds in the presence and absence of NaCl in the elution solvent were obtained. The experimental results indicate that the recoveries of target compounds had no obvious difference when NaCl concentration ranges from 0 to 5%. On basis of these results, NaCl was not used in the subsequent experiments.

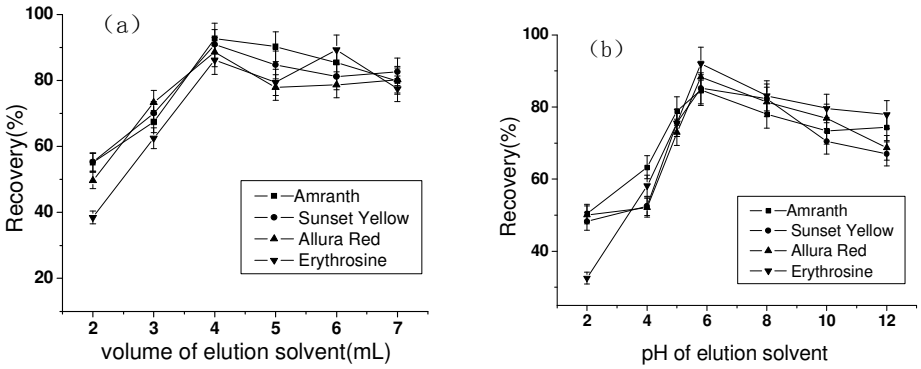


Fig.3 Influence of the volume (a) and pH value (b) of elution solvent on analyte recoveries. Spike level: 500 ng·g^{-1}, eluant: water.

3.3. Evaluation of the method

3.3.1. Linearity, LOD and matrix effects

The calibration equations of the spiked samples, linear range and limits of detection (LODs) are presented in Table 1. The working curves were constructed by plotting the peak areas (*Y*) measured versus the concentrations (*X*) of analytes. The results showed good linearity for all the analytes with correlation coefficients (R) higher than 0.999 5 in the range of 50-2 000 ng·g^{-1} for four colorants. The limits of detection (LODs) and quantification (LOQs) are determined as the lowest concentration yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively. So the linear regression equations and LOQs are appropriate to the goal of the proposed method.

The matrix effects (ME) is defined as the ratio response of analyte peak in the presence and absence of matrix [47]. Matrix effects were evaluated by comparing the slopes of two calibration curves of colorants standards and chilli elution. Depending on the decrease/increase in the percentage of the slope, %ME in the range between -20% and 20% can be considered as

insignificant because such variability is close to the repeatability RSD values [48]. The results show that matrix effects for four colorants were small suppression or enhancement, from -9.7% to +8.2% in chilli powder. Hence, calibration standards in methanol can be used for quantification of colorants in chilli powder without the need for matrix-matching.

Table 1 Calibration curves, correlation coefficients (r), limits of detections (LODs) of the method.

Analytes	Calibration curve[a]	r	Linear range (ng·g^{-1})	LODs (ng·g^{-1})
Amranth	Y= -315.80+47.05X	0.999 4	30-200 0	13.5
Allura Red	Y=774.62+33.13X	0.999 6	20-200 0	8.6
Sunset Yellow	Y=871.51+54.58X	0.999 6	20-200 0	8.9
Erythrosine	Y=-246.03+54.33X	0.999 5	30-200 0	12.3

[a] X denotes concentration as ng·g^{-1} and Y was the peak area.

3.3.2. Intra-day and inter-day repeatability

Repeatability was evaluated by determining target analytes in spiked chilli powder samples at the spiked concentration of 200 ng·g^{-1} and 500 ng·g^{-1}. The intra-day precision was determined by analyzing the samples five replicates in one day. The inter-day precision was achieved by analyzing the samples once a day in five consecutive days. The results are presented in Table 2 and the intra-day RSD were lower than 2.2% which is lower than 2.4% for inter-day analysis.

Table 2. Intra-day and inter-day repeatability

Analytes	Added (ng·g^{-1})	Inter-day repeatability RSD (n = 5) (%)	Intra-day repeatability RSD (n =5) (%)
Amranth	200	2.3	2.0
	500	1.7	1.9
Allura Red	200	2.2	2.1
	500	1.9	1.3
Sunset Yellow	200	2.4	1.8
	500	1.3	2.2
Erythrosine	200	1.1	1.5
	500	0.9	1.4

3.3.3. Stability

It is reported that dyes particularly synthetic dyes are known for their poor light fastness. Therefore, long-term stability of analytes in chilli powder during sample storage in the dark was evaluated. The spiked samples were prepared according to the method mentioned in Section 2.3 and then kept in sealed bottle at 4 ℃ in the dark. The resulting samples were analyzed in five replicates after 1, 3 and 5 weeks, respectively. The losses and RSD values listed in Table 3 range from 4.66 to -1.21% and 1.5 to 4.1%, respectively. It can be concluded that the colorants in chilli powder samples were stable in the dark for at least five weeks.

Table 3 The stability of the analytes in spiked Sample (n=5)

Added	Stored	Amranth	Allura Red	Sunset Yellow	Erythrosine

(ng/g)	time (week)	Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)
	1	3.05	2.5	2.36	2.2	1.56	3.0	2.02	3.5
200	3	2.97	2.1	3.89	3.0	3.45	3.1	1.80	3.1
	5	3.76	3.3	4.33	3.3	-0.87	2.5	2.95	1.5
	1	3.23	3.4	3.90	2.1	-1.21	2.6	3.27	3.0
500	3	4.66	4.1	4.02	3.2	3.65	3.2	4.26	2.7
	5	-1.02	1.9	3.11	2.6	4.32	3.6	4.31	2.9

[a]Loss (%) = $\frac{\text{Mean concentration of analyte in fresh samples(ng/g)}}{\text{Mean concentration of analyte in stored samples(ng/g)}} \times 100\text{-}100$

3.3.5. Analysis of samples and recovery

In order to validate applicability of the MSPD-HPLC method, three original chilli powder samples collected from the local markets were analyzed. No residue of analytes was observed in all samples. To assess the accuracy of the proposed method, recovery studies were also calculated by determining known amounts of analytes in spiked samples. The spiked samples were treated by the present method, and the extracts were analyzed by HPLC-UV. The chromatograms of blank and spiked chilli powder samples are shown in Fig.4. The amounts of the analyte in spiked samples were then measured. As can be seen in Table 4, the mean recoveries for all the analytes were in the range of 87.9-104.2% with RSD less than 3.3%, which demonstrated that the MSPD-HPLC method was reliable and could be used for the determination of amranth, allura red, sunset yellow and erythrosine in chilli samples. Because the colorants in the original samples were not detectable, the formula for recovery is:

$$\text{Recovery} = \frac{\text{Amount of analyte found in spiked sample(ng/g)}}{\text{Amount of analyte spiked in the sample(ng/g)}} \times 100\%$$

Table 4. Recoveries of the analytes in spiked samples (n = 3)

Analytes	Added (ng·g^{-1})	Founded (ng·g^{-1})	Recovery (%)	RSD (%)
Amranth	50.0	48.1	96.2	1.2
	100.0	95.0	95.0	2.0
	200.0	207.0	103.5	2.1
Sunset Yellow	50.0	45.9	91.8	2.6
	100.0	101.2	101.2	1.8
	200.0	177.1	88.6	2.4
Sllura Red	50.0	45.3	90.6	3.3
	100.0	95.1	95.1	2.2
	200.0	188.9	94.5	2.9
Erythrosine	50.0	52.1	104.2	2.7
	100.0	87.9	87.9	1.7
	200.0	185.3	92.7	1.9

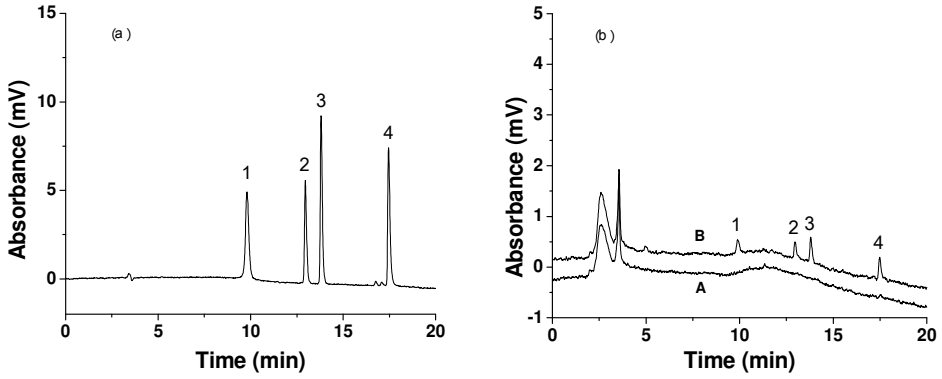


Fig.4. (a) Chromatograms of standard solution; (b) Chromatograms of blank (A) and spiked (B) samples. 1. Amaranth; 2. sunset yellow; 3. allura red; 4. erythrosine.

3.3.6. Comparison with other methods

The comparison of the MSPD-HPLC-UV method with other reported methods for determination of colorants in foods was shown in Table 5. Compared with the reference methods [30, 33, 35, 37], the present method is simpler, time-saving and organic solvent in the proposed method was used. In addition, satisfactory limits of detection in the present method was obtained, which demonstrated the good practicability of the proposed MSPD-HPLC-UV method.

Table 5. Comparison of MSPD with other methods

Analyte	Sample	Extraction method	Type and volume of extration solvent	LOD ($ng \cdot g^{-1}$)	Reference
Sudan I, II, III and IV	Egg yolk	MIM-MSPD-DLLME	4mL of methanol-water (1/1, v/v); 3mL of acetone-acetic acid (95/5,v/v)	2.3-6.1	37
4 banned dyes	Condiments	IL-based MSPD-HLLME	[C6mim][BF4] (120uL) 6 mL of water	6.7-26.8	15
Sudan I, II, III and IV	Sauces and Condiments	MSPD	4 mL of hexane 3 mL of acetonitrile	50-90	32
Sudan I, II, III and IV	Chilli foods	SPE	120 mL of acetonitrile	13.2-19.1	33
4 colorants	Chilli powder	MSPD	4 mL of water	8.6-13.5	Present work

4. Conclusion

A simple and green MSPD-HPLC-UV method for the simultaneous determination of amranth, allura red, sunset yellow and erythrosine in chilli powder samples has been developed. No organic solvent was used in the preparation of sample solution, and a clean and interference-free chromatogram was obtained for the sample matrices tested in this study just as water was used as extraction solvent. Compared with other extraction methods reported, the present method is much easier and more eco-friendly. Good results indicated that the present approach was a simple and green procedure to determine synthetic food colorants at trace level. It can also be extended to be

applied in other similar samples by varying the extraction conditions.

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Tables

Table 1 Calibration curves, correlation coefficients (r), limits of detections (LODs) of the method.

Analytes	Calibration curve[a]	r	Linear range ($ng \cdot g^{-1}$)	LODs ($ng \cdot g^{-1}$)
Amranth	Y= -315.80+47.05X	0.999 4	30-200 0	13.5
Allura Red	Y=774.62+33.13X	0.999 6	20-200 0	8.6
Sunset Yellow	Y=871.51+54.58X	0.999 6	20-200 0	8.9
Erythrosine	Y=-246.03+54.33X	0.999 5	30-200 0	12.3

[a] X denotes concentration as $ng \cdot g^{-1}$ and Y was the peak area.

Table 2. Intra-day and inter-day repeatability

Analytes	Added ($ng \cdot g^{-1}$)	Inter-day repeatability RSD (n = 5) (%)	Intra-day repeatability RSD (n =5) (%)
Amranth	200	2.3	2.0
	500	1.7	1.9
Allura Red	200	2.2	2.1
	500	1.9	1.3
Sunset Yellow	200	2.4	1.8
	500	1.3	2.2
Erythrosine	200	1.1	1.5
	500	0.9	1.4

Table 3 The stability of the analytes in spiked Sample (n=5)

Added (ng/g)	Stored time (week)	Amranth		Allura Red		Sunset Yellow		Erythrosine	
		Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)	Loss[a] (%)	RSD (%,n=5)
200	1	3.05	2.5	2.36	2.2	1.56	3.0	2.02	3.5
	3	2.97	2.1	3.89	3.0	3.45	3.1	1.80	3.1
	5	3.76	3.3	4.33	3.3	-0.87	2.5	2.95	1.5
500	1	3.23	3.4	3.90	2.1	-1.21	2.6	3.27	3.0
	3	4.66	4.1	4.02	3.2	3.65	3.2	4.26	2.7
	5	-1.02	1.9	3.11	2.6	4.32	3.6	4.31	2.9

[a] $\text{Loss}\ (\%) = \frac{\text{Mean concentration of analyte in fresh samples(ng/g)}}{\text{Mean concentration of analyte in stored samples(ng/g)}} \times 100\text{-}100$

Table 4. Recoveries of the analytes in spiked samples (n = 3)

Analytes	Added ($ng \cdot g^{-1}$)	Founded ($ng \cdot g^{-1}$)	Recovery (%)	RSD (%)
Amranth	50.0	48.1	96.2	1.2
	100.0	95.0	95.0	2.0
	200.0	207.0	103.5	2.1
Sunset Yellow	50.0	45.9	91.8	2.6
	100.0	101.2	101.2	1.8
	200.0	177.1	88.6	2.4
Sllura Red	50.0	45.3	90.6	3.3
	100.0	95.1	95.1	2.2
	200.0	188.9	94.5	2.9
Erythrosine	50.0	52.1	104.2	2.7
	100.0	87.9	87.9	1.7
	200.0	185.3	92.7	1.9

Table 5. Comparison of MSPD with other methods

Analyte	Sample	Extraction method	Type and volume of extration solvent	LOD ($ng \cdot g^{-1}$)	Reference
Sudan I, II, III and IV	Egg yolk	MIM-MSPD-DLLME	4mL of methanol-water (1/1, v/v); 3mL of acetone-acetic acid (95/5,v/v)	2.3-6.1	22
4 banned dyes	Condiments	IL-based MSPD-HLLME	[C6mim][BF4] (120uL) 6 mL of water	6.7-26.8	7
Sudan I, II, III and IV	Sauces and Condiments	MSPD	4 mL of hexane 3 mL of acetonitrile	50-90	19
Sudan I, II, III and IV	Chilli foods	SPE	120 mL of acetonitrile	13.2-19.1	20
4 colorants	Chilli powder	MSPD	4 mL of water	8.6-13.5	Present work

Figures

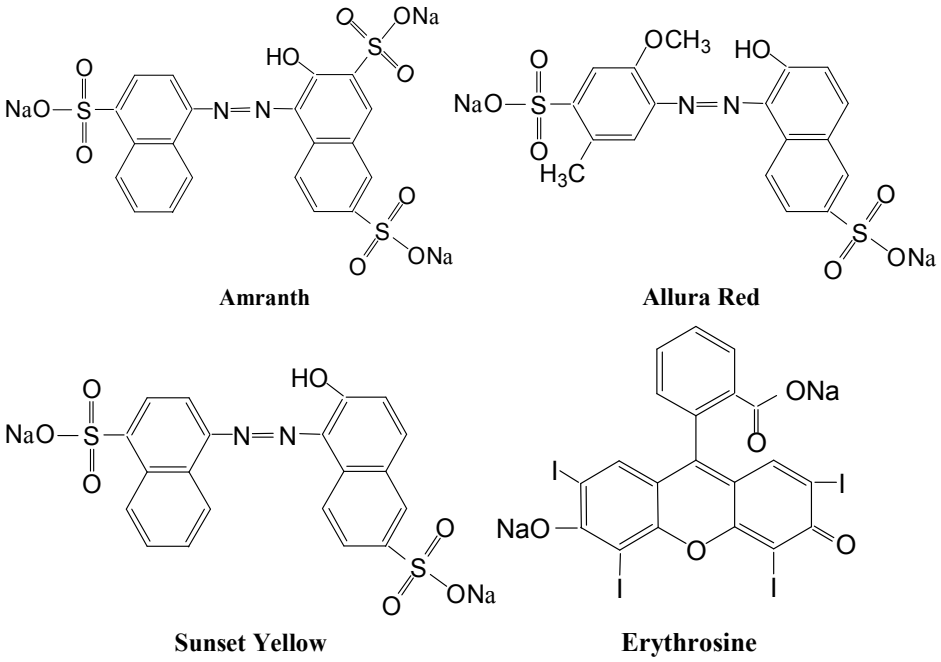

Fig.1 Chemical structure of analytes.

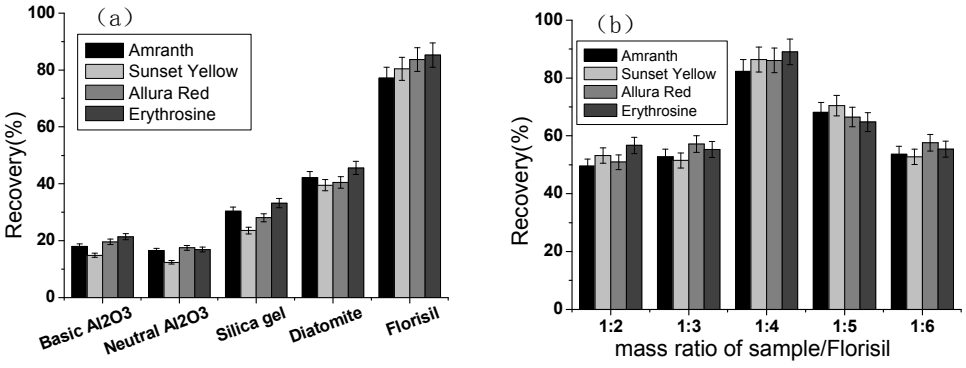

Fig.2 Influence of the sorbent (a) and mass ratios of sample/Florisil (b) on MSPD procedure. Spike level: 500 ng·g^{-1}, eluant: water.

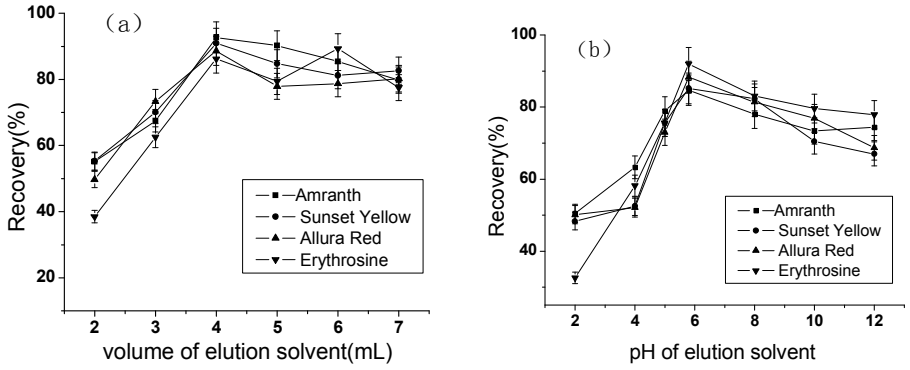

Fig.3 Influence of the volume (a) and pH value (b) of elution solvent on analyte recoveries. Spike level: 500 ng·g^{-1}, eluant: water.

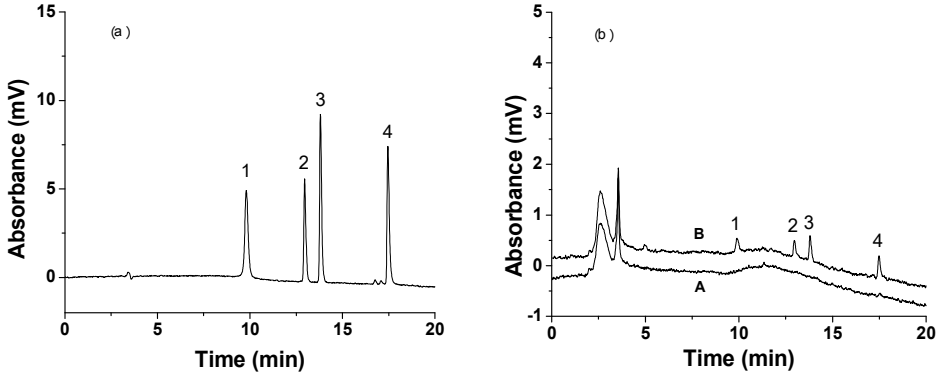

Fig.4. (a) Chromatograms of standard solution; (b) Chromatograms of blank (A) and spiked (B) samples. 1. Amaranth; 2. sunset yellow; 3. allura red; 4. erythrosine.