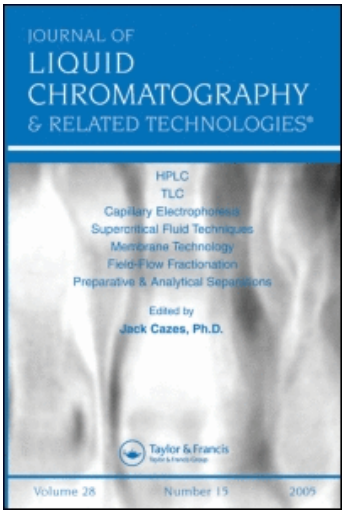

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□ *In this article, a novel method, microwave assisted micellar extraction coupled with high performance liquid chromatography has been developed for the determination of five anthraquinone derivatives (aloe-emodin, rhein, emodin, chrysophanol, physcion) in semen cassiae. Some important parameters, such as the pH value of the solution, microwave assisted micellar extraction time, extraction power, concentration of surfactant Genapol X-080 solution, liquid to solid ratio, and semen cassiae granularity were investigated. Optimum extraction conditions were obtained as follows: 0.1 g 180 μm particle size of semen cassiae, 10% Genapol X-080 (w/v), pH 3.0, 260 w microwave power, microwave assisted extraction time for 3 min, and liquid/solid ratio of 60:1 (mL/g). The method yields linear calibration curves in the concentration range of 1.0 to 40 μg/mL for target analytes, and the detection limits for the five anthraquinone derivatives were between 6.0 ng/mL and 39 ng/mL. The relative standard deviations (R.S.D.) were less than 1% (n = 6). In addition, the MAME method established in this article is demonstrated to be a simple, economic, and environmentally friendly alternative to conventional organic solvent extraction methods.*

Keywords genapol X-080, HPLC, micellar extraction, microwave assisted extraction, semen cassiae

INTRODUCTION

Semen cassiae, the dry mature seed of *Cassia obtusifolia L*, is a commonly used traditional Chinese herb. It is reported to have the pharmaceutical effect of clearing away liver-fire, a syndrome of traditional Chinese medicine, and improving visual acuity.[1] A variety of constituents have been isolated from semen cassiae, such as aloe-emodin, rhein, emodin, chrysophanol, physcion, and glucosides, which are accepted as the most important active components.[2]

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Methods commonly used for the determination of anthraquinone derivatives in semen cassiae are high performance liquid chromatography (HPLC),[3] capillary electrophoresis (CE),[4] and thin-layer chromatography (TLC).[5] Inevitably, extraction of the analytes is required in these methods. But, conventional methods usually use large amounts of toxic organic solvents and need relatively long time to extract the targets.

Micellar extraction is a kind of novel extraction method which utilizes micellar media as extractants. From an analytical point of view, one of the most important properties of surfactants is their good capacity to solubilize solutes of different types found in different environments.[6] In recent years, the use of microwave for extraction of constituents from plant materials has drawn tremendous research interest. Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X-ray and infrared rays in the electromagnetic spectrum,[7] unlike conventional heating that depends on a conduction-convection phenomenon, with eventually much of the heat energy being lost to the environment. In the case of microwave assisted extraction (MAE), heating occured in a targeted and selective manner with practically no heat being lost to the environment, as the heating occurred in a closed system. This unique heating mechanism can significantly reduce the extraction time.[8]

The combination of the MAE technique with micellar extraction has been carried out in our laboratory for the extraction of Tanshinones from Salvia Miltiorrhiza Bunge, which shows to be a simple, fast, low cost, easy handling, and non-toxic procedure.[9] In this article, the application potential of the microwave assisted-micellar extraction (MAME) has been further evaluated by employing non-ionic surfactant oligoethylene glycol monoalkyl ether (Genapol X-080) for the extraction of five anthraquinone derivatives in semen cassiae. To optimize the MAME step, the influences of pH and concentration of the surfactant solution, granularity of the plant powder, liquid/solid ratio, microwave power, and extraction time were all investigated.

EXPERIMENTAL

Instrumentation

All analyses were performed on a Shimadzu LC-10A liquid chromatograph (Shimadu, Japan) equipped with a solvent delivery pump, Shimadzu SPD-10A UV-VIS detector, and a 7125 injection valve with 20 mL loop. The chromatographic data were recorded and processed with N 2000 software (Zhejiang University, Hangzhou, China). A versatile plant pulverizer (Foshan, Guangdong, China) was used to make the plant materials into

powder. A KQ-250 ultrasonic generator (Kunshan Company, Jiangsu, China) was used to extract anthraquinone derivatives from the samples. Sieves (40 mesh/420 μm, 60 mesh/250 μm, 80 mesh/180 μm, 100 mesh/150 μm, and 140 mesh/105 μm) were used to sieve the semen cassiae powder. A high speed centrifuge was employed to centrifuge the sample solutions (LG 10-2.4A, Beijing, China). A WBFY-201 microwave chemical reactor (650 w, Gongyi, Henan, China) was used for the extraction.

Plant Materials

The semen cassiae herb was purchased from a local pharmaceutical store (Baoding, China). The dried plant materials were pulverized and sieved to produce samples with particle sizes of 40 mesh/420 μm, 60 mesh/250 μm, 80 mesh/180 μm, 100 mesh/150 μm, and 140 mesh/105 μm.

Chemicals and Reagents

All anthraquinone derivatives (Aloe-emodin, Rhein, Emodin, Chrysophanol, and Physcion) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The individual standard stock solution was prepared in methanol at a concentration of 80 μg/mL and stored at 4°C (Physcion was first dissolved in a small volume of 1,4-dioxane, then it was further diluted in methanol). Non-ionic surfactant oligoethylene glycol monoalkyl ether (Genapol X-080) was obtained from Fluka (USA) and used as received without further purification. Various concentrations (w/v) of aqueous surfactant solutions were weighed accurately and transferred into a 250 mL volumetric flask. Doubly distilled water was added and diluted up to the mark. Then the solution was diluted to get concentrations of 20%, 15%, 10%, 5%, and 1% (m/v). Methanol was of HPLC-grade and was obtained from KEMIO Company (Tianjin China). All the solvents and water samples were filtered through a 0.45 μm membrane before analysis.

Experimental Procedure

Extraction Procedure

Semen cassiae was accurately weighed and placed in a round-bottom flask. Genapol X-080 solution at a concentration level of 10% was added. The pH value of the solution was adjusted to 2.3 using H_3PO_4. The flask was capped and blended adequately, then it was placed in the microwave chemical reactor to assist extraction, the semen cassiae extract was centrifuged for 10 min, supernatant fluid was transferred, and diluted up to

8 mL with the Genapol X-080 solution of same concentration. Finally, the extract was filtered through a 0.45 μm filter and 5 μL of the supernatant was injected for HPLC analysis.

Comparison with Conventional Extraction Techniques

Extraction efficiency was compared between MAME and conventional extraction techniques, such as ultrasonic extraction (UAE) and static extraction at room temperature (ERT). For the comparison experiment, identical experimental conditions were used: sample amount 0.1 g, 180 μm particle size of semen cassiae, 10% Genapol X-080, liquid/solid ratio of 60:1(mL/g), but with a different extraction time: UAE 30 min., ERT 12 h., MAME 3 min.

HPLC Analysis

Separation was carried out on a Diamonsil C_{18} column (150 mm × 4.6 mm i.d., 5 μm). The mobile phase was a mixture of methanol-H_3PO_4 (85:15, v/v) and the flow rate was 1.0 mL/min, the detection wavelength was set at 254 nm. In this report, peaks in the chromatograms were identified by comparing the retention times and UV spectra with those of the authentic anthraquinone derivatives. Peak area was used for quantification. To avoid the potential influence of Genapol X-080 to the separation of anthraquinone derivatives, the column was flushed with methanol to completely elute Genapol X-080 after each day's work.

RESULTS AND DISCUSSION

Optimization of the Microwave-Assisted Extraction Conditions

To optimize the microwave-assisted micellar extraction of anthraquinone derivatives from the solid herbal materials, a number of experiments under different conditions were performed. The pH value of the solution, extraction time, microwave power, concentration of the surfactant solution, liquid/solid ratio, and granularity of the plant powder were all investigated and evaluated via yield of extracts (%, w/w):

$$\text{yield of extracts } (\%, \text{w/w}) = \frac{\text{amount of anthraquinone derivative extracted}}{\text{amount of herbal material}} \times 100\%$$

Effect of pH on the Extraction Efficiency of Semen Cassia

The original value of the extraction solution was 4.81. In the case of other conditions fixed, phosphoric acid was used to adjust the pH

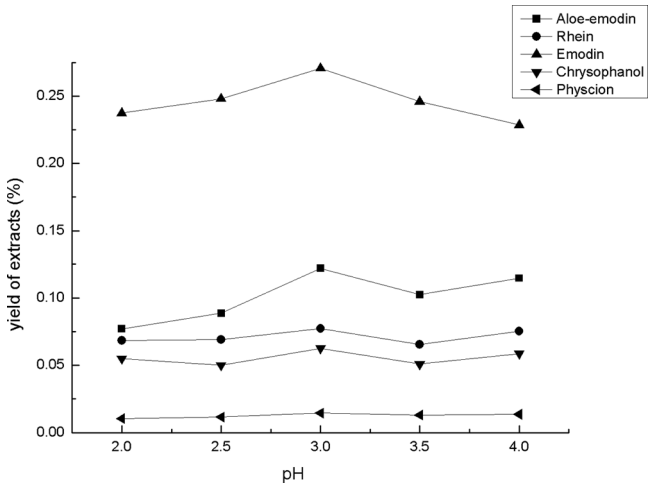

FIGURE 1 Effect of pH on the extraction efficiency of semen cassiae. Extraction conditions: 0.2 g semen cassiae, concentration of Genapol X-080: 10% (m/v), granularity: 200 μm, microwave power: 40%, liquid/solid ratio: 40 (mL/g).

between 2.0 and 4.0. As shown in Fig. 1, pH 3.0 was the optimum condition; the same pH value was chosen for the mobile phase. Phosphoric acid played the role of the ionization-inhibitor, so that the anthraquinone derivatives could be extracted in molecular forms.

Effect of Extraction Time on the Extraction Efficiency of Semen Cassiae

The influence of extraction time on the extraction efficiency of semen cassiae was studied by varying the extraction time from 0 to 10 min (1, 3, 5, 7, 10 min). As shown in Fig. 2, the extraction yield of rhein went up when the extraction time was prolonged from 1 to 5 min. After 5 min, the extraction yield declined. The physcion's extraction efficiency did not change much with the extraction time. As for other anthraquinone derivatives, the extraction efficiency reached the maximum value in 3 min and then began to decline. The possible reason for this phenomenon is that the temperature of the reactor becomes too high after long-time microwave reaction, and the structure of the component changes at high temperature. In the experiment, when the microwave extraction time was increased from 5 to 10 min, an intermittent heating method was employed to prevent overheating and loss of the sample solution. Considering the extraction efficiency, 3 min was chosen as the optimum extraction time.

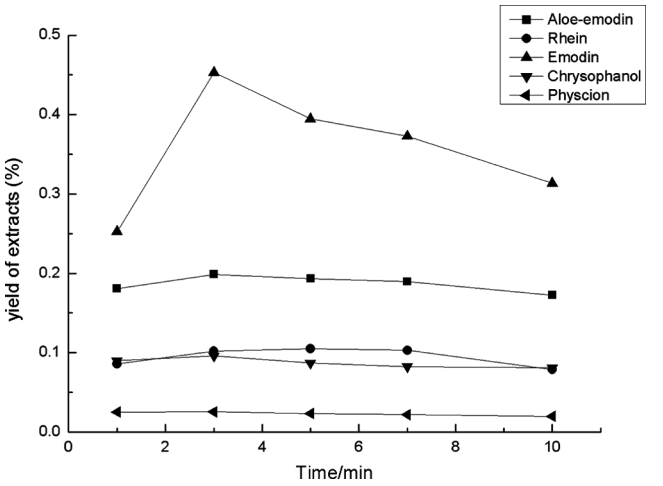

FIGURE 2 Effect of extraction time on the extraction efficiency of semen cassiae. Extraction conditions: 0.2 g semen cassiae, concentration of Genapol X-080: 10% (m/v), granularity: 180 μm, pH: 3.0, microwave power: 40%, liquid/solid ratio: 40 (mL/g).

Effect of Microwave Power on the Extraction Efficiency of Semen Cassiae

To evaluate the influence of microwave power on the extraction efficiency of semen cassiae, experiments were carried out by setting the microwave power at 10%, 20%, 30%, 40%, and 50% of the total power (650 w). As shown in Fig. 3, the extraction efficiency of emodin increased

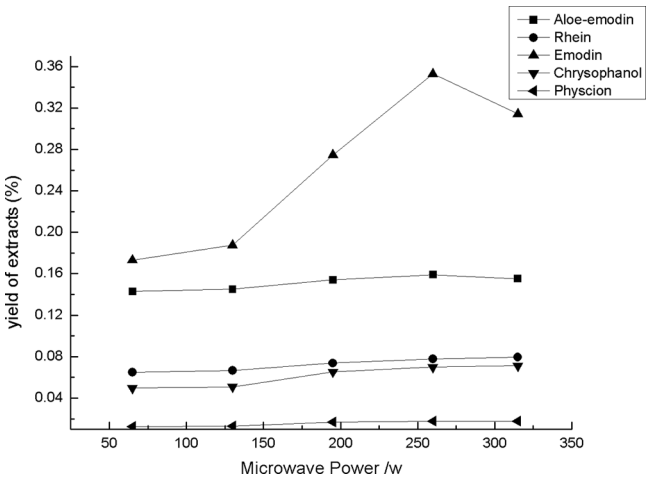

FIGURE 3 Effect of microwave power on the extraction efficiency of semen cassiae. Extraction conditions: 0.2 g semen cassiae, concentration of Genapol X-080: 10% (m/v), granularity: 180 μm, pH: 3.0, liquid/solid ratio: 40 (mL/g).

significantly; when the microwave power increased from 10% to 40%, then the extraction yield dropped. The extraction efficiency did not change much for the other four anthraquinone derivatives. As result, 40% was chosen as the optimum microwave power for the microwave-assisted micellar extraction of semen cassiae.

Effect of the Surfactant Concentration on the Extraction Efficiency of Semen Cassiae

The ability of the aqueous non-ionic Genapol X-080 solution in extracting anthraquinone derivatives may be related to the solubility-enhancement effect of the surfactant micelles. It can be seen from Fig. 4 that the amount of extracted anthraquinone derivatives increased greatly when the surfactant concentration increased from 1.0% to 10% (w/v). When the surfactant concentration rose to 20%, the extraction efficiency for all the anthraquinone derivatives changed slightly. Considering both the maneuverability and the cost, 10% was chosen as the optimum surfactant concentration for the extraction of semen cassiae for further studies.

Effect of Liquid/Solid Ratio on the Extraction Efficiency of Semen Cassiae

The liquid/solid ratio is the proportion of the extractant volume to the mass of herbal material. It is one of the factors influencing the extraction efficiency of semen cassiae. In this part, liquid/solid ratio of 20, 40, 60, 80,

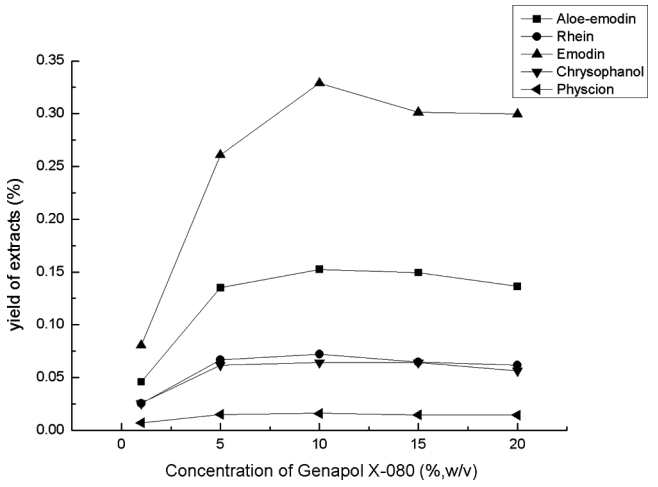

FIGURE 4 Effect of surfactant concentration on the extraction efficiency of semen cassiae. Extraction conditions: 0.2 g semen cassiae, granularity: 180 μm, pH: 3.0, liquid/solid ratio: 40 (mL/g).

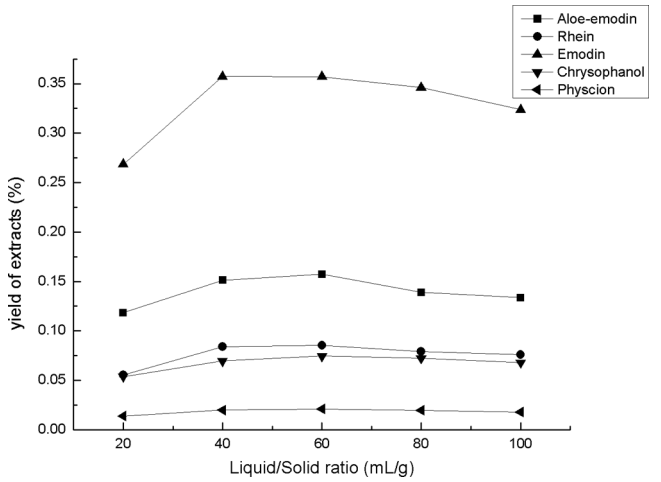

FIGURE 5 Effect of liquid/solid ratio on the extraction efficiency of semen cassiae. Extraction conditions: 0.1 g semen cassiae, concentration of Genapol X-080: 10% (m/v), granularity: 180 μm, pH: 3.0.

100 (mL/g) were tested to optimize the extraction procedure. It could be seen from Fig. 5 that the liquid/solid ratio of 60 was sufficient for semen cassiae to reach the highest extraction efficiency.

Effect of Granularity of the Plant Powder on the Extraction Efficiency of Semen Cassiae

Granularity is another important factor influencing the extraction efficiency. In our experiment, a series of granularity (40 mesh/420 μm, 60 mesh/250 μm, 80 mesh/180 μm, 100 mesh/150 μm, and 140 mesh/105 μm) were compared. The results showed that the extraction efficiency reaches the highest value when plant powders of 180 μm were used (Fig. 6).

Comparison of Microwave Extraction with Conventional Extraction Techniques

Extraction efficiency was compared between microwave assisted micellar extraction (MAME) and conventional extraction techniques such as ultrasonic assisted extraction (UAE) and static extraction at room temperature (ERT). In addition, MAME was also compared with the refluxing extraction method recommended by Chinese Pharmacopoeia (2005 edition) (CPEM).[10]

Figure 7 shows that (MAME) has the highest extraction efficiency. MAME not only provides high extraction efficiency, but also costs short

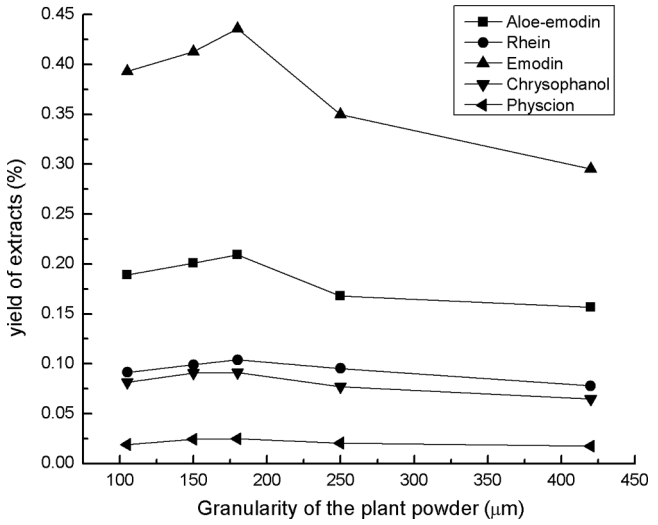

FIGURE 6 Effect of granularity of the plant powder on the extraction efficiency of semen cassiae. Extraction conditions: 0.1 g semen cassiae, concentration of Genapol X-080: 10% (m/v), pH: 3.0, liquid/solid ratio: 60 (mL/g).

time. Furthermore, it is less labor intensive. Therefore, MAME is an alternative extraction technique for fast extraction of anthraquinone derivatives from semen cassiae.

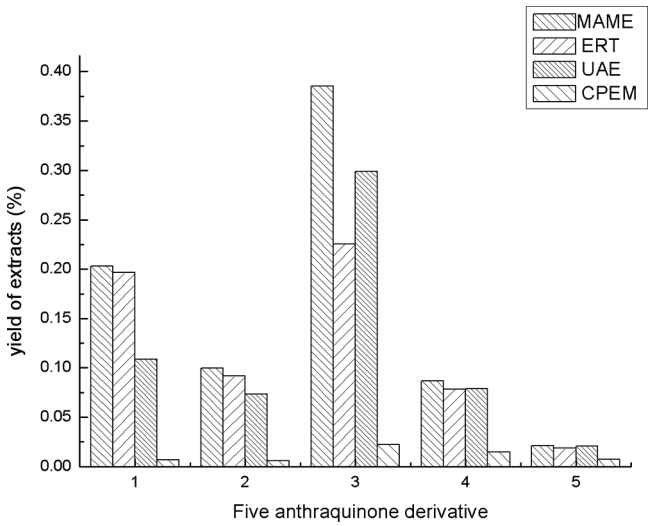

FIGURE 7 Comparison of MAME and conventional extraction techniques. 1. Aloe-emodin, 2. Rhein, 3. Emodin, 4. Chrysophanol and 5. Physcion. Experimental conditions: Genapol X-080: 10% (m/v), 0.1 g semen cassiae, liquid/solid ratio: 60 (mL/g). ERT: 12 h, UAE: 30 min, MAME: 3 min.

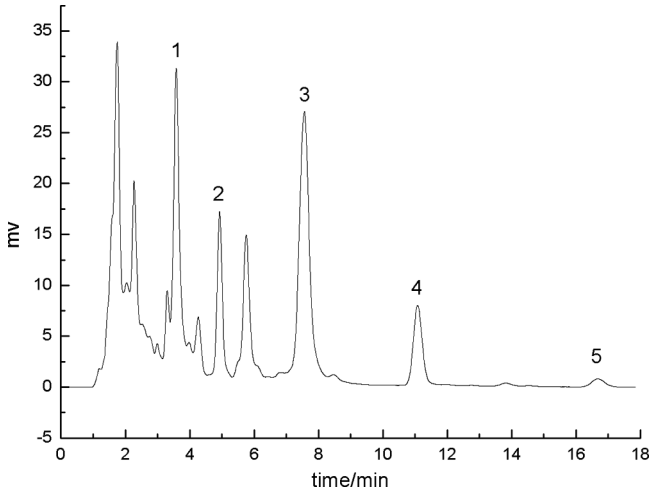

FIGURE 8 HPLC chromatograms of anthraquinone derivatives extracted from semen cassiae. 1. Aloe-emodin, 2. Rhein, 3. Emodin, 4. Chrysophanol and 5. Physcion. Experimental conditions: 0.1 g semen cassiae, pH 3.0, concentration of Genapol X-080: 10% (m/v), granularity: 180 μm, pH: 3.0, liquid/solid ratio 60:1.

Analysis of Anthraquinone Derivatives in Semen Cassiae by HPLC

Figure 8 shows that the five anthraquinone derivatives were completely separated from each other, and other coexisting components in the extracts of semen cassiae did not interfere with the detection of the anthraquinone derivatives except for the case of Aloe-emodin.

Evaluation of MAME Method

The method yields linear calibration curves in the concentration range between 1.0 and 40 μg/mL for target analytes. All calibration graphs were plotted based on linear regression analysis of the integrated peak area

TABLE 1 The Regression Equations, Correlation Coefficients, Linear Ranges, and the Limit of Detection (LOD) for the Analysis of the Anthraquinone Derivatives ($n=3$)

Compounds	Linear Regression Equation	Linear Range (μg/mL)	R	LOD (μg/mL)
Aloe-emodin	$Y=-2.33\times10^{4}+3.78\times10^{4}X$	1.0∼40	0.9995	0.0061
Rhein	$Y=-5.47\times10^{3}+3.92\times10^{4}X$	1.0∼40	0.9998	0.02
Emodi	$Y=-2.06\times10^{4}+3.78\times10^{4}X$	1.0∼40	0.9995	0.039
Chrysophanol	$Y=-2.17\times10^{4}+3.99\times10^{4}X$	1.0∼40	0.9995	0.011
Physcion	$Y=-1.73\times10^{4}+3.06\times10^{4}X$	1.0∼40	0.9996	0.006

X denotes concentration (μg/mL) of the Semen Cassiae, Y denotes peak area.

TABLE 2 RSD of Peak Area and Retention Time

Compound	Retention Time R.S.D.(%)	Peak Area R.S.D.(%)
Aloe-emodin	0.55	0.12
Rhein	0.93	0.13
Emodi	0.28	0.18
Chrysophanol	0.71	0.23
Physcion	1.00	0.47

(Y) versus concentration (μg/mL, X) of the anthraquinone derivatives in the standard solution at five different concentrations (each concentration injected three times). The regression equations, correlation coefficients, linear ranges, and the limit of detection (LOD) for the analysis of the anthraquinone derivatives are shown in Table 1.

Under optimum conditions, RSD of peak area and retention time were less than 1.0% for the five anthraquinone derivatives extracted from Semen Cassiae sample, as shown in Table 2.

CONCLUSIONS

In this article, the microwave-assisted micellar extraction method was developed for the separation and simultaneous determination of anthraquinone derivatives from semen cassiae, prior to HPLC analysis. Experimental results indicate that the combination of the microwave assisted extraction and micellar extraction makes the method rapid, simple, and environmental-friendly, which may be a good alternative to the traditional extraction techniques.

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REFERENCES

1. Ou, M. *Chinese-English Manual of Common-Used Herbs in Traditional Chinese Medicine*, Guangdong Science and Technology Publishing House: Guangdong, China, 1992; 243.
2. Chen, Q. D.; Xu, R.; Xu, Z. N.; Cen, P. L. Progress in Studies of Active Constituents of Anthraquinones and Their Biological Activities from Semen Cassiae. *Chin. J. Mod. Appl. Pharm.* **2001**, *32*, 858.

3. Yu, C.; Lan, H. M.; Wang, Y. Determination of Anthraquinone Compounds in Semen Cassiae by RP-HPLC. *Primary J. Chin. Materia Medica.* **2002**, *16*, 8.
4. Zheng, W. J.; Chen, X. G.; Jia, W.; Qiu, M. F.; Xu, Z. H. Determination of Emodin and Aloe-Emodin in Semen Cassiae and Its Tea Preparations by High Efficiency Capillary Chromatography. *Chin. Tradit. Herbal Drugs*, **2004**, *35*, 874.
5. Zang, J.; Wang, T.; Zhang, C. Z. Extraction and TLC Test of Anthraquinones in Semen Cassiae. *J. Dalian Inst. Light Ind.* **2006**, *25*, 43.
6. Ferrera, Z. S.; Sanz, C. P.; Santana, C. M.; Rodriguez, J. J. S. The Use of Micellar Systems in the Extraction and Pre-Concentration of Organic Pollutants in Environmental Samples. *Trends in Anal. Chem.* **2004**, *23*, 469.
7. Letellier, M.; Budzinski, H. Microwave Assisted Extraction of Organic Compounds. *Analysis*, **1999**, *27*, 259.
8. Mandal, V.; Mohan, Y.; Hemalatha, S. PHCOG REV.: Review Article Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharm. Rev.* **2007**, *1*, 7.
9. Shi, Z. H.; Wang, Y.; Zhang, H. Y. Combination of Microwave Assisted Micellar Extraction and Liquid Chromatography for Determination of Cryptotanshinone, Tanshinone I, and Tanshinone IIA in Salvia Mitiorrhiza Bunge. *J. Liq. Chrom. Relat. Tech.* **2009**, *32*, 698.
10. The Pharmacopoeia Committee of China. *The Chinese Pharmacopoeia, Part I*, The Chemical Industry Publishing House: Beijing, China, 2005; 191.