JOURNAL OF
CHEMICAL EDUCATION

# A Novel Green Sample Pretreatment Method Column-Free Matrix Solid-Phase Dispersion Extraction: Application in a High-Performance Liquid Chromatography Experiment for Undergraduate Chemistry

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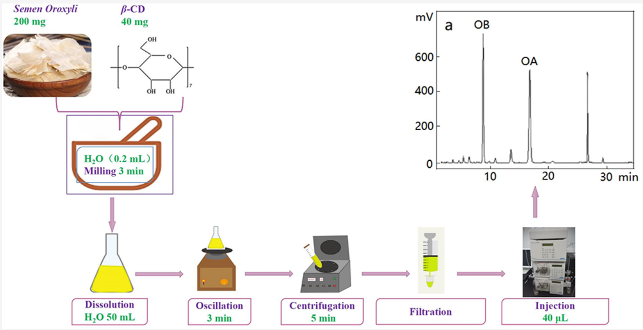
Cite This: *J. Chem. Educ.* 2024, 101, 3369−3376



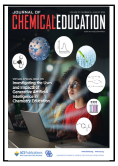
**ABSTRACT:** Here, we describe a student experiment that uses a novel column-free matrix solid-phase dispersion (FMSPD) extraction combined with high-performance liquid chromatography (HPLC) for quantitative determination of oroxin A (OA) and oroxin B (OB) from *Semen Oroxyli* (the seeds of *Oroxylum indicum* (L.) Vent.). Compared with traditional sample pretreatment methods, which are time-consuming and complex and use a large amount of organic solvents, the new FMSPD method is fast, safe, simple, and easy to operate. It does not use organic solvents and is convenient for undergraduate teaching experiments. By comparing retention times of the OA and OB standards at the same chromatographic conditions, students were able to identify these two components present in *Semen Oroxyli* and to quantify their contents using calibration curves of OA and OB. In addition, the same *Semen Oroxyli* sample was extracted using the traditional ultrasonic-assisted extraction method reported in the literature and determined using HPLC. Any significant difference between the two methods was evaluated using *F*- and *t*-test statistical methods. The results indicated that the new sample preparation can be used instead of the traditional ultrasonic-assisted extraction method. The experiment provides an opportuni students to be exposed to a new extraction technique to enrich their horizons. At the same time, students can learn some new theoretical knowledge such as solubilization, host−guest interaction, and inclusion complexes while also consolidating their learning of the key theoretical concepts including the liquid chromatography separation mechanism, sample preparation and cleanup method for complex samples, and statistical analysis of the data.

**KEYWORDS:** *third-year undergraduate, column-free matrix solid-phase dispersion extraction, HPLC, quantitative analysis, statistical analysis of data, Green Chemistry*

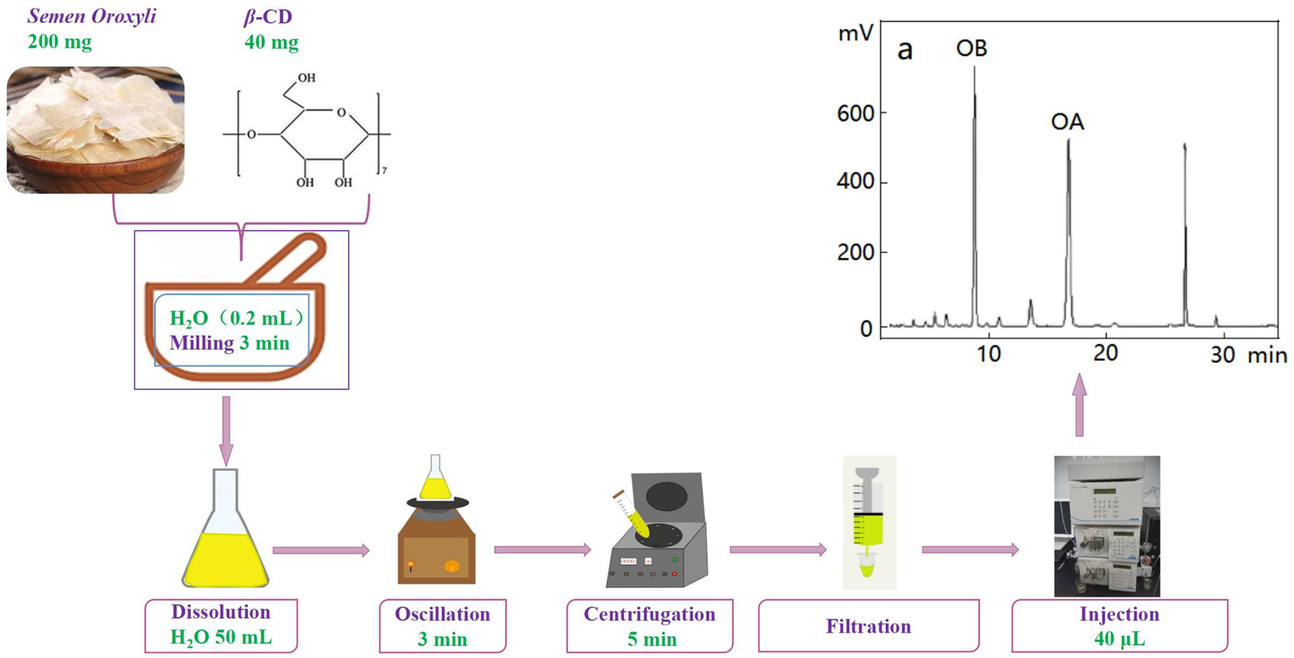
## ■ INTRODUCTION

High-performance liquid chromatography (HPLC) is an important branch of chromatography. It has become an important separation and analysis technology in chemistry, medicine, industry, agronomy, commodity inspection, legal inspection, and other disciplines.[1] An HPLC experiment, as a typical instrumental analysis experiment, is widely used in the experimental teaching of analytical chemistry at the undergraduate level.[2−4] HPLC needs to be combined with some sample pretreatment techniques to realize the separation and detection of actual samples. In undergraduate laboratory experiments,[5,6] traditional sample pretreatment methods are usually used, such as solvent extraction,[7,8] solvent reflux extraction[9,10] and ultrasonic-assisted extraction.[11,12] These methods take a long time, use a large amount of organic solvents, and require a relatively cumbersome operation. Therefore, from the point of view of saving time and safety, many universities use standard substances instead of actual samples for HPLC analysis. For example, our HPLC experiment for the third-year chemistry students at Guizhou University used reversed-phase separation and UV−vis detection to separate the aniline isomer standards. If standard compounds are employed as the target of separation, the absence of sample pretreatment steps will lead to limited comprehension and proficiency in the practical application of HPLC for complex samples. Because sample preparation is necessary to analyze complex samples using HPLC, can we find a faster, simpler, safer, and environmentally friendly sample pretreatment method? We once conducted a

**Received:** November 10, 2023
**Revised:** June 18, 2024
**Accepted:** June 18, 2024
**Published:** June 29, 2024



**Scheme 1. Experimental Flow Diagram**



questionnaire survey on sample pretreatment methods among 40 undergraduates, and less than 5% of them knew new sample pretreatment techniques such as matrix solid-phase dispersion extraction, liquid−liquid microextraction, solid-phase microextraction,[13] but had not actually used them. Thus, the introduction of some new, green sample pretreatment techniques can enrich the content of undergraduate experimental teaching and broaden students' perspectives. At the same time, the characteristics of being fast, safe, and easy to operate the new sample preparation methods make them convenient as an undergraduate teaching experiment.

Matrix solid-phase dispersion (MSPD) is a promising approach for the pretreatment of samples. Through a grinding process, the sample is thoroughly dispersed into the solid phase and subsequently transferred to a syringe barrel. The resulting mixture exhibits characteristics of being partially dry and free-flowing, allowing for subsequent elution using one or more solvents, akin to conventional solid-phase extraction (SPE).[14] The grinding process involves the application of mechanical shear forces as well as hydrophilic and hydrophobic interactions between the sample and the sorbent. These interactions facilitate complete destruction and dispersion of the sample into the solid phase.[15] The primary benefits of MSPD are enhanced sample extraction efficiency and purification before analysis. Nevertheless, the incorporation of SPE for isolating the desired compounds introduces several drawbacks, including a time-intensive and intricate column packing procedure, as well as the need for a substantial quantity of hazardous organic reagents.[16] Thus, the method is still not simple, fast, or safe enough to be used in sample pretreatment for undergraduate HPLC experiments. Our research group reported a novel green sample pretreatment method called column-free matrix solid-phase dispersion (FMSPD) for the extraction of three flavonoids from the seeds of *Oroxylum indicum* (L.) Vent.[17] In that study, cucurbit[8]uril was used as the sorbent of FMSPD to selectively extract three flavonoid compounds and transfer them to an aqueous solution for direct determination using HPLC−UV based on the recognition interaction of cucurbit[8]uril toward the compounds. This method does not require column loading nor does it use organic solvents and has the characteristics of being simple, fast, safe, and easy to operate. Cucurbit[8]uril is difficult to synthesize and expensive; therefore, it is not suitable for undergraduate teaching. Therefore, we chose *β*-cyclodextrin (*β*-CD), which is cheaper, safe and nontoxic and also has a cavity structure, to replace cucurbit[8]uril as the sorbent for FMSPD, and designed a new sample pretreatment method for HPLC experiment in undergraduate teaching.

*β*-CD is a macrocyclic compound polymerized from seven glucose units, with a hydrophobic cavity and an external hydrophilic symmetrical cylinder structure.[18] This structure enables *β*-CD to form stable host−guest inclusion complexes with guest molecules through hydrophobic interaction, hydrogen bonding, electrostatic interaction, etc.[19,20] Meanwhile, because of the hydrophilicity of *β*-CD, the inclusion complexes formed can improve the solubility of hydrophobic guest molecules in water.[21,22] Compared with other cyclodextrin isomers, the aqueous solubility of *β*-CD is relatively low; however, it is cheaper and easy to form host−guest inclusion complexes with guest molecules to improve the solubility of the insoluble guest molecules. *β*-CD is commonly used as a drug carrier for many small drug molecules and has been widely used in pharmaceutical applications.[23,24]

*Semen Oroxyli*, the seeds of *Oroxylum indicum* (L.) Vent., is a commonly used traditional Chinese medicinal herb. It exhibits analgesic, apoptotic, antitussive, anti-inflammatory and antioxidant activities and has been widely used to treat cough, acute pharyngitis and respiratory disorders.[25] Chemical and pharmacological studies have indicated that oroxin A (OA) and oroxin B (OB) are the major bioactive flavonoids used as the index components for the quality control of *Oroxylum indicum* (L.) medicinal materials because of their high levels in *Semen Oroxyli*.[25]

In this work, *β*-CD was used as a solid-phase dispersible extractant to grind together with the *Semen Oroxyli* powder.

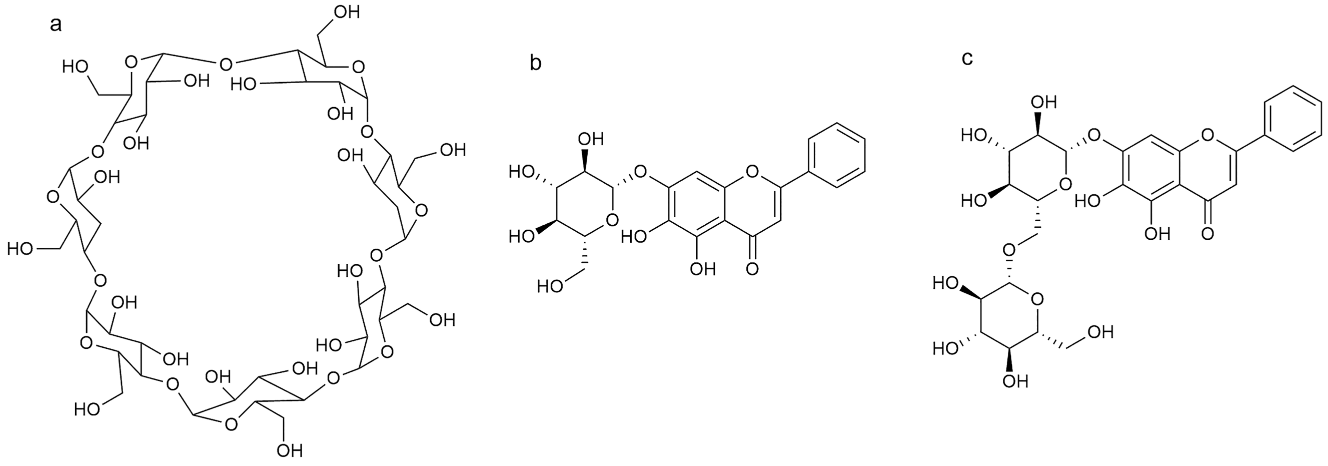


**Figure 1.** Chemical structures of $\beta$-CD (a), OA (b), and OB(c).

During the grinding process, $\beta$-CD exhibits selective extraction of OA and OB, leading to the formation of the corresponding inclusion complexes. These inclusion complexes enhance the water solubility of hydrophobic OA and OB, enabling direct dissolution in water followed by filtration for subsequent injection into HPLC to determine their contents. The whole experimental process is shown in Scheme 1. This sample preparation is different from MSPD in that it does not require column loading and does not use organic solvents for elution, making it easier to operate, simple, fast, and environmentally friendly. Green analytical chemistry (GAC) emerged from green chemistry in 2000, which concerns the role of analytical chemists in making laboratory practices more environmentally friendly. The 12 principles of GAC proposed by Namiesnik et al. provide essential guidelines for making analytical laboratories greener. Its contents include reduction in the use of reagents and energy, elimination of waste, risk and hazard, the use of natural reagents, and increasing operator safety.[26] The new sample pretreatment method well embodies the purpose of the 12 GAC principles, and at the same time, the experimental process can also convey the concept of GAC to students and cultivate their environmental awareness.[27] The proposed new HPLC laboratory experiment based on the sample pretreatment of FMSPD was used by third-year undergraduates in the Department of Chemistry at Guizhou University. To give students a better understanding of the new sample pretreatment method, the ultrasonic-assisted extraction method reported in the literature was also compared to better highlight the advantages of the new method. To our knowledge, there are no publications reporting the analysis of OA and OB from *Semen Oroxyli* using FMSPD coupled with the HPLC method in the *Journal of Chemical Education*. The pedagogical aims of this experiment are the following:

- To expose students to some new cutting-edge technologies, broaden their horizons, and cultivate their scientific research interest.
- To understand the application of HPLC in practical sample determination.
- To understand FMSPD concepts such as extraction principle, operation procedure, and influencing factors.
- To understand the qualitative identification of OA and OB present in *Semen Oroxyli* and their quantification utilizing calibration curves.
- To learn to judge significant differences between different sample preparation techniques using statistical methods.
- To introduce some new theoretical knowledge concepts and principles including host−guest interactions, inclusion complexes, solubilization, etc. for enriching students' knowledge reserves.
- To strengthen and consolidate theoretical knowledge such as sample preparation, chromatography separation and data analysis processing.

## ■ EXPERIMENTAL SECTION

### Reagents

OA and OB standards were purchased from TaiTan Corporation (ShangHai, China) with purities >98%. HPLC-grade acetonitrile and methanol were obtained from Kemiou Corporation (Tianjin, China) and used as the mobile phase. A Milli-Q Water Purification System from Millipore Corporation (Bedford, MA, USA) was used to prepare ultrapure water. $\beta$-CD was purchased from TaiTan Corporation (ShangHai, China) with puritiy >98%. Samples of *Semen Oroxyli* were purchased from Anhui in China. The structures of $\beta$-CD, OA and OB are shown in Figure 1.

### Preparation of Standard Solutions

Standard stock solutions were prepared by dissolving each compound in methanol at a final concentration of 1 mg/mL and further diluted with methanol to obtain working solutions of different concentrations. The standard stock solutions were stored at −18 °C and standard working solutions were stored at 4 °C in a refrigerator.

### Sample Preparation by FMSPD Method

The *Semen Oroxyli* dry crude materials were smashed into powder using a crusher (Guiyang, China) or a mortar. A total of 200 mg of the pulverized *Semen Oroxyli* sample, a 200 $\mu$L of water, and 40 mg of $\beta$-CD as a solid phase dispersant were mixed in an agate mortar using a pestle for 3 min to obtain a semidry and homogeneous mixture. The homogeneous mixture was placed in a 100 mL conical flask, and 50 mL of water was added and swirled to blend for 3 min. A 6 mL portion of the mixture was removed and transferred into a 10 mL centrifuge tube, followed by centrifugation at 5000 rpm for 5 min, the supernatant was filtered through a 0.22 $\mu$m microporous filter membrane, and 40 μL of solution was injected into HPLC for analysis.

### Sample Preparation by Ultrasonic-Assisted Extraction Method

A conventional ultrasonic-assisted extraction method was adopted for comparison, and the detailed procedure used according to the literature method[28] was as follows: 200 mg of the powdered sample was extracted with 40 mL of methanol in an ultrasonic bath at 40 °C for 40 min. The extract was centrifuged at 4000 rpm for 10 min at room temperature. The residues were re-extracted and centrifuged using the same procedure. The supernatants were combined and diluted with methanol to 100 mL. After filtering through a 0.22 μm microporous filter membrane, 40 μL of solution was injected into the HPLC for analysis.

### Optimization of Extraction Conditions of FMSPD

The method of optimization adopts a single-factor investigation, which fixes other variables while changing only one factor. Factors for optimization include the grinding time, the amount of β-CD, and the amount of water added.

### Chromatographic Analysis

HPLC analysis was performed on an Elite P230 II liquid chromatographic instrument equipped with a UV/vis spectroscopic detector (Dalian, China). A Rheodyne 7725i manual sample injector with a 20 μL loop was used for the sample injection. A Hypersil ODS column (4.6 × 150 mm, 5 μm) was purchased from Eilit Corporation (Dalian, China) and used to separate OA and OB. An isocratic mobile phase, composed of 20% ultrapure water with 0.1% acetic acid and 80% HPLC grade acetonitrile, was utilized at a flow rate of 0.8 mL/min. The detection wavelength was 268 nm, and the column oven was set to room temperature.

## ■ HAZARDS

Lab coats, safety glasses, and gloves must be worn at all times in the laboratory. Organic solvents were used under a fume hood. Methanol and acetonitrile are known irritants to the skin and eyes and are flammable. The centrifuge must be stopped before the samples can be taken out. A waste container is connected to the HPLC to ensure proper disposal of solvents.

## ■ RESULTS AND DISCUSSION

The laboratory experiment is for third-year chemistry students as a comprehensive chemistry experiment (8−10 h). Students worked in groups of two, and five groups formed a large group to share an HPLC instrument. We have two HPLC instruments that can perform experiments with 20 students at a time. Forty students from the different classes of the third-year chemistry major chose this experimental course and were divided into four large groups to complete the experiment. Various lectures of at least 1−2 h were presented before the lab experiment to introduce the principle, experimental process, instruments, and factors affecting extraction efficiency (see the Supporting Information for details). Students were required to complete a prelab section to promote understanding of the whole method, including sample pretreatment and chromatography analysis (see the Supporting Information). The experiment was divided into four parts: (1) qualitative identification of OA and OB present in *Semen Oroxyli* and the construction of calibration curves of OA and OB standards; (2) optimization of FMSPD extraction conditions, including grinding time, the amount of β-CD, and the amount of added water; (3) determination of real samples and calculation of the contents of OA and OB in a *Semen Oroxyli* sample; and (4) extraction of the same samples using literature-reported ultrasonic-assisted extraction, followed by HPLC analysis.

During this experiment, OA and OB standards are analyzed, as shown in Figure 2a, while the HPLC chromatogram of the extract of the *Semen Oroxyli* sample pretreated by FMSPD is shown in Figure 2b. The specific compounds in the sample can be identified and quantified by comparison with the standards of OA and OB at the same chromatography conditions. As shown in Figure 2a, the retention time of the OA standard was 16.52 min and that of the OB was 8.58 min. It was also clear that these two components were present in the sample (Figure 2b) by comparison. Five mixed solutions of OA and OB standards were analyzed using HPLC from the lowest to highest concentration. Then, the calibration curves of the two compounds were plotted using linear regression analysis of peak heights against concentrations (Figure 3). The calibration curves for the other three groups can be found in the Supporting Information. The purpose of this part of the experiment is to let students know how the components in a complex sample are qualitatively identified and how the standard curve is measured and drawn.

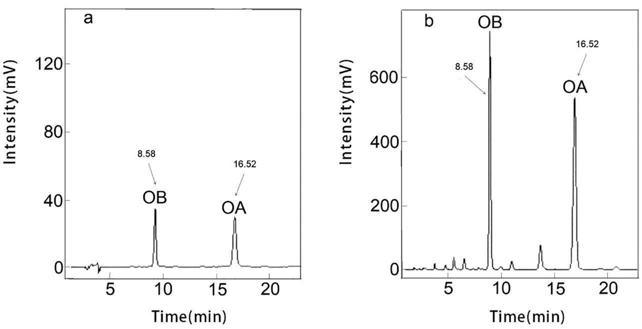


**Figure 2.** HPLC chromatogram of OA, OB standards (a) and the extract of the *Semen Oroxyli* sample pretreated by FMSPD method (b)

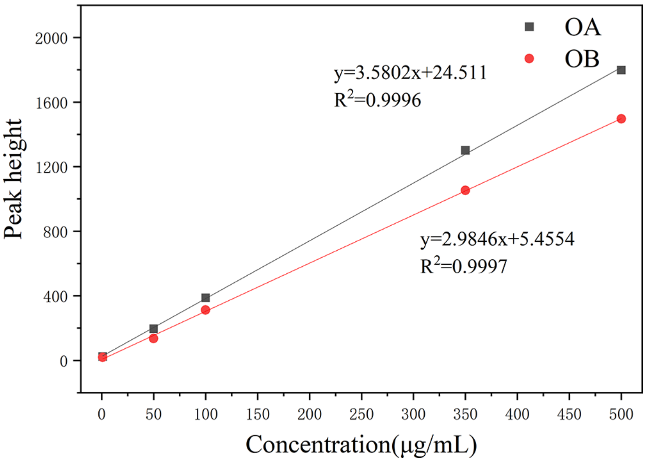


**Figure 3.** Calibration curves using OA and OB standards information.

The second part of the experiment is the optimization of the FMSPD extraction conditions. For any sample pretreatment method, it is necessary to optimize the extraction conditions

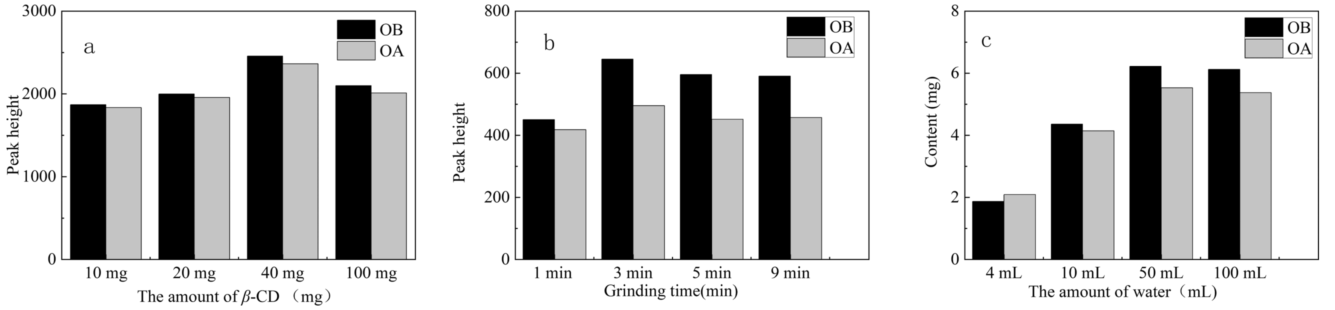


**Figure 4.** Optimization of extraction conditions of OA and OB compounds by FMSPD including the amount of β-CD (a), the grinding time (b), and the volume of water (c).

involved to achieve the best extraction results. The method of optimization generally adopts a single-factor investigation, which fixes other variables while changing only one factor. Before the experiment, through the experiment preview and instructor's explanation, the students should understand that the principle of FMSPD extraction is based on the formation of the host−guest inclusion complexes between β-CD and OA and OB compounds, and the formation of inclusion complexes promotes the solubility of the hydrophobic target compound (OA and OB) in aqueous solution, to achieve the purpose of direct detection of target compounds in aqueous solution. Among them, the formation of host−guest inclusion complexes in the grinding process is very critical. The instructor can explain to the students that the inclusion complexes have been confirmed through the LC-MS diagram of the extract of *Semen Oroxyli* pretreated by FMSPD method (Figure S 1), which can help students better understand the principle of FMSPD extraction (see the Supporting Information for details). From the schematic diagram of the extraction process (Scheme 1), we selected three important factors that affect the extraction efficiency, namely, the amount of dispersion sorbent (β-CD), the grinding time, and the amount of water added. Each factor was set at four levels, and different groups of students could investigate different influencing factors at the same time and come up with the best value for each influencing factor (see Supporting Information for details). Finally, the optimum conditions of the whole extraction process can be obtained after the summary. This step can cultivate the spirit of cooperation among students and make the students understand the necessity of optimizing extraction conditions. Figure 4 shows the optimization results for the three factors. The optimal extraction conditions were as follows: 40 mg of β-CD, grinding time of 3 min, and 50 mL of water. When examining the influence factor of the amount of water added, the instructor should tell students not to judge the result by the sizes of the chromatographic peak heights, but by the contents of the component. This is because the more water added, the lower the concentration and thus the smaller the peak heights. Therefore, the effect of volume dilution should be considered, and the component contents should be used as the basis for evaluation.

After the optimization of extraction conditions is completed, the third part of the experiment is to provide actual samples for students to determine. The samples were extracted under optimal conditions and further determined using HPLC to obtain the contents of OA and OB present in the *Semen Oroxyli* samples. Five replicate samples were measured to investigate the repeatability of the method, and the contents of the OA and OB samples are shown in Table 1. The results of the other three groups were presented in the Supporting Information for details.

**Table 1. Contents of OA and OB in the Five Samples Extracted by FMSPD Method**

| Sample number | OA | | OB | |
|---|---|---|---|---|
| | Concentration (μg/mL) | Content (mg/g) | Concentration(μg/mL) | Content (mg/g) |
| 1 | 224.6 | 56.15 | 235.5 | 58.87 |
| 2 | 232.1 | 58.03 | 241.7 | 60.14 |
| 3 | 239.8 | 59.95 | 223.7 | 55.93 |
| 4 | 241.9 | 59.30 | 240.6 | 60.28 |
| 5 | 237.2 | 57.09 | 239.4 | 60.14 |
| Mean(mg/g) ± RSD% | 58.50 ± 2.8 | | 59.25 ± 2.9 | |

To help students obtain a better understanding of the new FMSPD method, students were asked to use the traditional ultrasonic-assisted extraction method reported in the literature[28] to extract the same samples and determine the contents using HPLC in the fourth part of the lab experiment. Four replicate samples were determined by this method (Table 2), and the results were compared with those of the FMSPD extraction. As shown in Table 1 and Table 2, the measured results of the two methods were comparable.

**Table 2. Contents of OA and Ob in the Four Samples Extracted by Ultrasonic-Assisted Extraction Method**

| Sample number | OA | | OB | |
|---|---|---|---|---|
| | Concentration (μg/mL) | Content (mg/g) | Concentration (μg/mL) | Content (mg/g) |
| 1 | 213.1 | 53.26 | 220.5 | 55.13 |
| 2 | 218.2 | 54.54 | 235.9 | 58.97 |
| 3 | 225.2 | 56.29 | 224.8 | 56.21 |
| 4 | 224.4 | 56.11 | 230.4 | 57.61 |
| Mean(mg/g) ± RSD% | 55.05 ± 3.7% | | 56.98 ± 4.8% | |

As for whether there were significant differences between the two methods, we asked students to use *F*- and *t*-tests to judge and explain what results they could obtain in the report after the experiment, and designed a table to compare the two extraction methods in terms of HPLC chromatograms, extraction time, whether organic solvents were used, and extraction equipment. The templates and writing requirements for lab reports are described in detail in the supporting documents. This part of the content can better help students to

**Table 3. Significant Difference between the Two Methods Evaluated by *F*- and *t*- tests ($n_1$ = 5, $n_2$ = 4, $\alpha$ = 0.05)**

| Methods | OA | | | OB | | | | |
|---|---|---|---|---|---|---|---|---|
| | Content/ (mg/g) ± RSD/% | $F_{calculated}$ | $t_{calculated}$ | Content/ (mg/g) ± RSD/% | $F_{calculated}$ | $t_{calculated}$ | $F_{4,3}$ | $t_{0.05,7}$ |
| ultrasonic-assisted extraction -HPLC | 55.05 ± 3.7 | 2.08 | 0.91 | 56.98 ± 4.8 | 1.84 | 1.92 | 9.12 | 2.36 |
| FMSPD- HPLC | 58.50 ± 2.8 | | | 59.25 ± 2.9 | | | | |

consolidate their theoretical knowledge of statistical analysis and understand the superiority of the new method. In the students' report (Table 3), the obtained *F* values for OA and OB were all below the critical *F* value ($F_{4,3}$ = 9.12 at the 95% confidence level), suggesting no significant difference in precision between the two methods (alpha = 0.05). Furthermore, the calculated *t* values were all lower than the critical table value ($t_{0.05,\,7}$ = 2.36), indicating that there was no significant difference in results when utilizing either the traditional ultrasonic-assisted extraction method or the novel FMSPD extraction method. The FMSPD method using *β*-CD as an adsorbent can be used as an alternative method for the determination of these compounds in *Semen Oroxyli*.

However, some students reported significant differences between the two extraction methods and concluded that there were systematic errors between them (see the Supporting Information for details). This is to encourage students to further discuss the causes of errors and ways to eliminate them in the lab report (see the Supporting Information for details). If they find a reason and are interested in doing it again, they may be allowed to make it up at another time after class after discussing it with the instructor.

Figure 5 shows HPLC chromatograms of the two extraction methods. As can be seen from Figure 5, the chromatogram of the new FMSPD extraction method was cleaner, indicating that the FMSPD extraction method has a good selectivity. Table 4 shows a comparison of the different aspects of the two extraction methods. Through these comparisons, students reported that the new method was faster, safer, simpler, and environmentally friendly. However, some students also pointed out that the optimization of extraction conditions took a lot of time, which was not as simple as the traditional method. Should we optimize every time? In response to this question, students are told that every extraction method needs to be optimized, and traditional methods are no exception. After the optimization of extraction conditions, it can be fixed as a mature analysis method and does not have to be optimized every time, so that the sample preparation can fully reflect the characteristics of being simple, fast, green, and effective for the new extraction method.

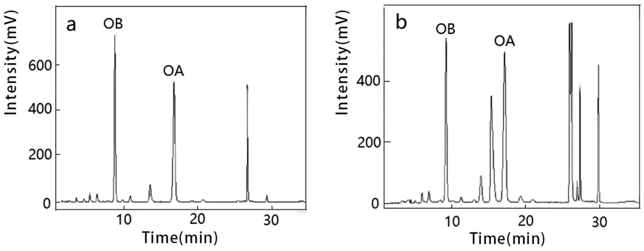


**Figure 5.** Comparison of HPLC chromatographs of the two extraction methods (a: FMSPD method; b: ultrasonic-assisted extraction method).

**Table 4. Comparisons of the Two Extraction Methods**

| | ultrasonic-assisted extraction | column-free matrix solid-phase dispersion extraction |
|---|---|---|
| Organic solvent | 100 mL methanol | no |
| Sample handing time | 80 min | <15 min |
| Equipment | ultrasonic bath | agate mortar |
| Operation | Relatively complex | simple |
| Safety | Relatively safe | safe |

Currently, 40 students have conducted the lab experiment, and all students completed the experiments within the time limit and without too many operational difficulties. In addition, the laboratory reports that were submitted showed that most students (85% obtained an A, 13% obtained a B and 2% obtained a C) had achieved the learning objectives laid out for the laboratory experiment. Based on an anonymous postlab survey (see the Supporting Information) and the laboratory reports, what students do best in these set goals is to consolidate the theoretical knowledge they have learned, such as chromatographic separation, sample preparation, data analysis processing, etc. As the students said, through this experiment, this knowledge became more tangible, and we can better understand and grasp it. At the same time, the analysis process of complex samples by HPLC was also clearer. For the new extraction method, the students showed great interest and recognition, and completed the experiment well. However, it was difficult for the students who did not have a good foundation to understand some new knowledge points involved, such as host—guest interaction, inclusion complexes, solubilization, etc. After all, supramolecular chemistry is an optional course they have not taken yet.

This experiment can also be performed as a typical single laboratory session (3−4 h) for the third-year chemistry students in the spring semester. Because of time constraints, the calibration curves may be prepared in advance, and the experiment to optimize extraction conditions is omitted. According to the optimization results of this experiment, the students will be directly asked to prepare and determine the samples under the optimal conditions to ensure that the whole experiment can be completed within the specified time. Forty-nine students were divided into four large groups to complete the experiment in this way, and their specific experimental results were exhibited in the lab report spreadsheet 3 (see the Supporting Information for details). We were more specific in our guidance to the students based on the previous experimental experience this time, so the students were able to complete the experiment within the designated time frame and achieved good academic results, with 86% of them earning an A grade while the remaining 14% received a B grade. To sum up, whether it is a comprehensive experiment (8−10 h) or a typical single laboratory session (3−4 h), the proposed method can meet the requirements of undergraduate teaching. Meanwhile, the comprehensive experiment will be continued in the fall semester for those senior students who have a strong scientific interest and are eager to improve their laboratory ability, but the number is limited to 20−30 students.

## CONCLUSION

We introduced a new simple, fast, safe, effective, and green sample pretreatment technique for undergraduate HPLC experiments. The combination of sample pretreatment and HPLC enables students to better grasp the application of HPLC to the determination of complex matrix samples. This analysis process includes preprocessing of the sample, qualitative and quantitative analysis of the components to be tested, and report writing. The determination of actual samples not only consolidates students' theoretical knowledge they have learned such as chromatographic separation, sample pretreatment, data analysis, etc. but also develop their relevant laboratory skills. The sample pretreatment method introduced is simple, fast, safe, and does not use organic solvents, which makes it particularly suitable for use in undergraduate teaching. Of course, the new sample pretreatment method is not limited to HPLC experiments. It may be used in combination with gas chromatography, fluorescence spectrophotometer, or UV−vis spectrophotometer for potential applications such as drug analysis, pesticide residue detection, or organic pollutants detection, which may provide additional advantages and enable students without HPLC access to benefit from the pedagogical opportunities. Moreover, the introduction of scientific research results into undergraduate teaching can enable students to access new knowledge and ideas and help them develop innovative thinking and scientific research interests.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.3c01163.

Instructor notes (PDF)
Instructor notes (DOC)
Detailed student experimental procedures and photos of experimental results (PDF)
Detailed student experimental procedures and photos of experimental results (DOC)
Example of a submitted lab report (PDF)
Example of a submitted lab report (DOC)
Example of a submitted lab report (PDF)
Example of a submitted lab report (DOC)
Example of a submitted lab report spreadsheet (XLSX)
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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was supported by Guizhou Science and Technology Foundation- ZK[2021] General 054, the National Natural Science Foundation of China (NO.21665005, NO. 22164006), and 2022 Reform Project of Teaching Content and Curriculum System in Higher Education of Guizhou Province - Teaching Reform of Inorganic Chemistry Course for Chemistry Majors (GZJG202206).

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