

# Constructing a Dynamic Material Perspective of Natural Medicines through Inquiry-Based Experiments: A Case Study of Analyzing Pre- and Post-Processing Components of *Panax ginseng* and *Panax quinquefolius* by UPLC

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**Abstract:** *The primary objective of the Natural Medicinal Chemistry experiment course is to help students establish a systematic understanding of the “material basis—bioactive function” relationship. However, traditional teaching has long been plagued by the issues of “emphasizing theory over techniques” and “focusing on results while neglecting processes,” making it difficult for students to develop transferable complex problem-solving skills. Guided by constructivist theory, this study designed an inquiry-based experimental teaching method for undergraduate students majoring in traditional Chinese medicine-related disciplines. The method integrates chromatography-spectroscopy techniques as the main framework, covering herbal pretreatment, isolation and purification of active components, structural identification, and quantitative analysis. Teaching effectiveness was evaluated through exploratory case studies. The results demonstrate that the application of chromatography-spectroscopy technology not only enhances students’ technical operational skills but also fosters the formation of a trinity material perspective of “holistic view-dynamic view-systematic view” in Chinese materia medica and natural medicines.*

**Keywords:** Natural medicinal chemistry, Experimental teaching reform, Chromatography-spectroscopy technology, Material perspective, Constructivism, Inquiry-based experiments, Systematic cognition.

## 1. Introduction

Natural Medicinal Chemistry is a crucial compulsory course for majors such as pharmacy and traditional Chinese pharmacy, holding a key position in the pharmaceutical education system. The main task of this course is to reveal the material basis for the prevention and treatment of diseases by natural medicines, elucidate their mechanisms of action, and provide a scientific basis for drug quality control and new drug research and development [1]. With the rise of the “return to nature” and “green movement” globally, natural medicines, as treasures of traditional medicine, are receiving increasing attention and recognition from modern medicine. However, natural medicinal chemistry research faces unique challenges: the inherent contradiction between the systemic nature of the material composition of natural medicines and the reductionist approach of modern chemical analysis methods. This contradiction directly impacts the teaching philosophy and methods of natural medicinal chemistry.

In traditional Natural Medicinal Chemistry experimental teaching, most experiments aim at “verifying known structures” as the endpoint. Students only need to repeat established steps to obtain the expected compound structure. This teaching model causes most students to fall into the learning dilemma of “seeing the trees but not the forest,” making it difficult to form a systematic understanding of the material basis of natural medicines. Although techniques like chromatography and spectroscopy have become increasingly sophisticated in scientific research, these advanced analytical techniques have not been fully integrated into undergraduate teaching scenarios due to various limitations such as long

experimental cycles, expensive equipment, and technical difficulty. This lag causes the technical content covered in undergraduate classrooms to fall short of meeting the demands of students’ further studies and the requirements for cultivating talents in the modernization of Traditional Chinese Medicine, necessitating urgent solutions through teaching reform.

The inherent contradiction in Natural Medicinal Chemistry essentially reflects the difference between reductionism and systems theory in the philosophy of science. Analytical methods guided by reductionism decompose complex systems into simple parts for study, focusing on the structure and properties of single components in natural medicines. However, this paradigm often overlooks the holistic characteristics of natural medicines constituted by active components. In recent years, research findings indicating that natural active components exert their effects as aggregates or other “systems” suggest that, in some cases, drug activity is not determined by a single compound. This discovery challenges the reductionist research paradigm and will guide researchers and educators in natural medicines to shift their thinking from reductionism to systems theory.

Modern educational theory, particularly constructivist learning theory [2], provides an important theoretical basis for the reform of Natural Medicinal Chemistry experimental teaching. This theory emphasizes that knowledge is not passively received but is a personal cognitive system actively constructed by learners in authentic contexts. Based on this concept, this study proposes a reform idea of orderly construction through “technique-context-thinking”: using

chromatography-spectroscopy techniques as a link to deeply integrate the holistic cognition of the active fractions or components of natural medicines into the entire experimental teaching process, guiding students to elevate from simple “identification of structure types” to “construction of a systematic material perspective,” thereby providing support for the analysis of complex pharmacodynamic mechanisms and the discovery of quality control markers.

## 2. The Complexity of the Active Material Basis of Natural Medicines and the Key Points / Difficulties in Knowledge Construction

The efficacy of natural medicines and their preparations does not stem from the isolated action of a single chemical component, but rather from the overall effect produced by the synergistic or antagonistic interactions of multiple active components through multiple targets and pathways [3, 4]. This complexity of the material basis is first manifested in the diversity of chemical composition: a single medicinal herb itself is a complex system containing hundreds or even thousands of components such as alkaloids, flavonoids, saponins, and polysaccharides, with interaction networks existing among different components. More critically, the relationship between the material basis of natural medicines and their pharmacodynamic functions is not a simple one-to-one correspondence. One component may act on multiple targets, while the realization of a pharmacological effect often requires the participation of multiple components, thus forming complex correlations of “multi-component, single-effect” or “multi-component, multi-effect.” This network characteristic of composition and function poses a significant challenge to the linear reductionist research paradigm of traditional analytical chemistry — “isolate-identify-determine activity” — when elucidating the holistic mechanism of action of natural medicines, and also dictates that its knowledge system inherently possesses a high degree of comprehensiveness and systematicity.

Faced with a complex cognitive object, the acquisition of knowledge cannot rely solely on passive indoctrination and mechanical verification but requires active construction. This view aligns with the core ideas of constructivist learning theory. This theory posits that learning is not simply transferring knowledge from the outside to the inside, but rather that learners actively construct their understanding of new knowledge based on existing experiences and cognitive structures, in authentic and complex situations, through processes of assimilation and accommodation [5, 6]. In learning Natural Medicinal Chemistry, students should not merely stay at the level of memorizing compound structures and activities. Instead, under teacher guidance, they should fully participate in the entire process from herbal pretreatment, activity-guided isolation, to compound structure identification and dose-effect relationship studies. During this process, learners need to integrate multidisciplinary knowledge from chemistry, biology, analytical chemistry, etc., repeatedly interact, conflict, and integrate new information—such as chromatographic retention behavior, spectral or mass spectrometric fragment features, and bioactivity data — with their existing cognitive schemas of “compound structure-property.” Ultimately, they form a transferable systematic cognitive model concerning the components of a

specific natural medicine, their interrelationships, mechanism of action, and quality control.

From the learner’s perspective, especially for undergraduates new to Natural Medicinal Chemistry, there are numerous cognitive difficulties in the process of constructing a systematic material perspective. These directly determine the focus and difficulty for teachers in instructional design and implementation. Students’ cognitive difficulties are mainly manifested in the following three aspects [7-8]: First, there is a disconnect between macroscopic efficacy and microscopic structure. Students find it hard to effectively link spectral or mass spectrometric data obtained from instrumental analysis, or microscopic molecular structures, with the macroscopic therapeutic effects of the whole drug, easily falling into the trap of identifying structures for the sake of identification. Second, faced with a vast amount of sometimes contradictory information on components and activities, students generally lack the awareness and ability to integrate information, not knowing how to sort and organize this information, leading to fragmented knowledge or even confusion. Finally, the leap from reductionist thinking to systems thinking is a cognitive leap in itself. Most students are accustomed to finding a single correct answer, so they feel confused and uncomfortable when confronted with the dynamic changes and synergistic effects of multiple components in natural medicines.

Therefore, the key points and difficulties for teachers lie in how to create effective teaching situations and scaffolds to help students overcome cognitive barriers. The main focus and efforts should be directed towards the following aspects: (1) Teaching should not be about directly providing conclusions, but about designing driving tasks. For example, instead of directly stating that “*Coptidis Rhizoma* mainly contains berberine,” pose a question like “How would you discover the material basis for the antibacterial activity of *Coptidis Rhizoma*?” This prompts students to actively connect techniques and knowledge in the process of problem-solving. (2) Teachers need to become organizers of students’ cognitive structures, skillfully using metaphors, diagrams, flowcharts, and other tools to visually transform techniques like chromatography and spectroscopy into “eyes” for students to observe the material world. This helps students establish a layer-by-layer analytical model of “herb → extract → fraction → component → single compound,” and systemic thinking that traces back from “single structure” to “holistic function.” (3) The greatest difficulty is guiding students to reflect on their cognitive processes through process evaluation and feedback, focusing on their thinking development from isolated knowledge points to forming a knowledge network, thereby truly achieving the transformation from passive memorizers to active constructors.

## 3. The Significance of Experimental Teaching for Constructing the Material Perspective of Natural Medicines

The material perspective, as an important component of scientific literacy, reflects a learner’s systematic understanding of the composition, organization, characteristics, and evolutionary patterns of matter in nature. In the discipline of Natural Medicinal Chemistry, this cognition is specifically manifested as a comprehensive

understanding of the complex relationships among the multiple chemical components, spatial configurations, and physiological effects within natural medicines. Experimental teaching, with its operational, contextual, and research-oriented characteristics, plays an irreplaceable role in helping students establish a cognitive system of matter. When teaching activities revolve around the discovery of active components, systematically demonstrating the complete research process of “chemical structure – method screening – information processing – mechanism investigation,” theoretical concepts can be transformed into actionable cognitive practices, making the experimental classroom a practical field for constructing the material perspective.

### 3.1 Experimental Teaching as a Practical Foundation for Constructing the Material Perspective

Experimental teaching provides students with a learning experience integrating perception, operation, and reflection, building a bridge connecting macroscopic phenomena with the microscopic world. During the extraction, separation, and identification of natural components, students engage in a series of operations—smelling herbal odors, observing color changes, precise weighing, adjusting pH, optimizing elution gradients—transforming abstract concepts from textbooks into tangible direct experiences. This multi-sensory learning approach effectively activates students’ deep cognitive processing, helping them understand the intrinsic link between the physicochemical properties of natural components and their molecular structures [9].

### 3.2 Triggering Cognitive Conflict and Conceptual Change in the Material Perspective

Cognitive conflicts embedded in the experimental teaching environment are important opportunities to drive students’ conceptual change and deepen their material perspective. When students encounter “anomalous phenomena”—such as significantly fluctuating extraction efficiency using the same herb, solvent, and equipment—the standard answers learned in traditional theory classes fail. This forces students to break out of the comfort zone of rote memorization and return to the complex system composed of multiple variables to find the root of the phenomenon. This process of “equilibrium disruption – explanation reconstruction” effectively guides students from mechanical imitation towards deep thinking about the intrinsic relationships among experimental variables, serving as a “gateway” for transforming their understanding of natural product material composition from a simple linear system to a nonlinear complex system.

### 3.3 Social Construction Fostered by Collaborative Group Work

Experimental teaching inherently possesses the attributes of social construction. In group collaboration, students engage in social interactions such as debating plans, comparing data, and evaluating results, constantly experiencing collisions between different perspectives. This experience within a community of inquiry practice not only promotes students’ metacognitive monitoring abilities but also helps them cultivate the spirit of teamwork.

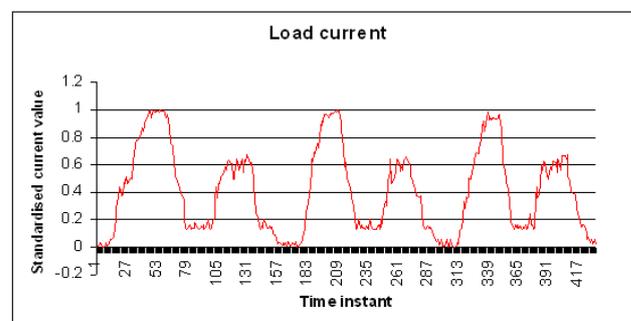


Figure 1: Testing data- load current (amperes)

### 3.4 Progressive Open Design: Matching Cognitive Capacity with Task Complexity

The educational function of experimental teaching is not automatically realized upon completion of the experimental operations; its effectiveness fundamentally depends on whether the task complexity is precisely matched with the cognitive capacity. If the task is too simple, students remain at the level of procedural operation, making it difficult to cultivate systematic scientific thinking. If the task is too complex and lacks necessary experiential support, students are prone to cognitive overload and frustration. Therefore, selecting appropriate teaching materials and formulating reasonable teaching methods based on the knowledge base and cognitive ability characteristics of undergraduates is particularly important in constructing the material perspective.

Hence, this study advocates a “progressive open” design principle for experimental teaching. In the introductory stage, scaffolding is built by designing semi-open tasks, such as providing a specific herb and target component and asking students to independently optimize extraction process parameters. This aims to help students initially establish a basic mapping relationship between “variable – result – mechanism.” In the capability enhancement stage, fully open tasks are designed. For example, faced with a natural mixture of unknown composition, students are challenged to independently or collaboratively design an activity-guided isolation scheme, comprehensively apply chromatography and spectroscopy techniques for component identification, correlate the findings with activity data, and finally integrate them into a complete evidence chain.

Through this spiraling cognitive training, experimental teaching can shed its traditional role as an auxiliary to theoretical courses and transform into a core driver for knowledge integration, methodological practice, and scientific thinking cultivation, ultimately helping students firmly establish a systematic material perspective of natural medicines.

## 4. Case Study on Teaching Reform: Constructing the Material Perspective of Natural Medicines Assisted by Ultra-Performance Liquid Chromatography

### 4.1 Research Background

This study program provides students with several optional

“micro-projects,” requiring them to complete independent inquiry within a specified timeframe. One of the projects is “Comparison of the Effects of Steaming Processing on the Composition of Rare Ginsenosides in *Panax ginseng* and *Panax quinquefolius*.” *Panax ginseng* (Renshen) is renowned as the “king of herbs,” and the distinctive efficacy of Hongshen (red ginseng) obtained after processing is closely related to the conversion of primary ginsenosides into rare ginsenosides [10]. Steaming *Panax quinquefolius* (Xiyangshen) can alter its cold property, thereby expanding its clinical application range [11]. This topic helps students construct a holistic and dynamic view of the active substances in Chinese/natural medicines, while cultivating their abilities in observation, analysis, comparison, and awareness.

## 4.2 Task Design

This selected “micro-project” belongs to the fully open task within the enhancement stage of the “progressive open” design. The group members are all fourth-year undergraduate students, with guidance from the instructor, teaching assistants, and laboratory technicians. In this “micro-project,” the designed tasks mainly include inquiry questions posed by the teacher, as well as questions that interest the students:

Task 1: Which components should be selected as indicators for quantification? How do the changes in these indicators reflect the overall dynamic changes of rare ginsenosides during the steaming process?

Task 2: What is the mechanistic basis for this pattern? (Students can accomplish this by literature review or experimental design). How can this pattern be described intuitively and quantitatively?

Task 3: What are the similarities and differences in the changes of rare ginsenoside composition between *Panax ginseng* and *Panax quinquefolius* before and after processing? What implications do these findings have for research and production?

## 4.3 Implementation Process

### 4.3.1 Experimental Materials

Instruments: Ultra-Performance Liquid Chromatography system (Waters Corporation, ACQUITY UPLC H-Class); High-speed universal pulverizer (Zhejiang Yili Industry & Trade Co., Ltd., QE400); Analytical electronic balance (Tianjin Tianma Hengji Instrument Co., Ltd., TD1002A); High-frequency numerical control ultrasonic cleaner (Kunshan Hechuang Ultrasonic Instrument Co., Ltd., KH300TDB); Centrifuge (Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd.)

Samples and Reagents: *Panax ginseng* (produced in Fusong County, Jilin Province), *Panax quinquefolius* (produced in Fusong County, Jilin Province), Methanol, Ginsenoside standards (Chengdu Pufei De Biotech Co., Ltd.; Batch numbers: Rg5: 21121403, Rh4: 23092503, Rk1: 22111403, Rk3: 23033003).

### 4.3.2 Preparation of Processed Ginseng Products

Take 0.6 kg of *Panax ginseng*/*Panax quinquefolius*, divide into three portions, rinse separately, and allow to stand in a moist state for 24 h. Place each portion into a steamer and steam for 2 h. After steaming, dry the samples in an oven for 72 h. Pulverize the dried processed herbal slices into a fine powder that can pass through a No. 4 sieve and set aside for later use.

### 4.3.3 Preparation of Test Solutions

Take 1 g each of the processed product powder and raw *Panax ginseng*/*Panax quinquefolius* powder obtained in section 3.3.2, add to conical flasks. Add 10 mL of methanol to each flask, seal, and sonicate (800 W, 40 kHz) in an ultrasonic cleaner twice, 60 min each time. Transfer the extracts to 50 mL centrifuge tubes, centrifuge at 3000 rpm for 3 min. Collect the supernatant and filter through a 0.22  $\mu\text{m}$  microporous membrane to obtain the filtrate, which serves as the test solution.

### 4.3.4 Preparation of Standard Solutions

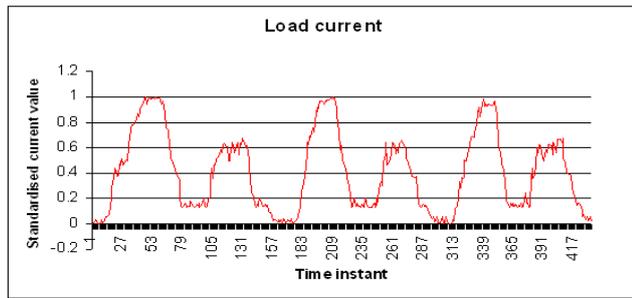
Accurately weigh appropriate amounts of rare ginsenoside Rk3, Rh4, Rk1, and Rg5 reference standards, place into separate 2 mL brown volumetric flasks, dissolve with methanol, and prepare stock solutions at concentrations of 0.292, 0.502, 0.905, 1.315, 0.554, 0.549, 0.642, and 0.939 mg/mL, respectively. Transfer 0.2 mL of each stock solution into a 2 mL EP tube, add 1 mL of methanol, vortex thoroughly, centrifuge at 13000 rpm for 10 min, collect the supernatant, and filter through a 0.22  $\mu\text{m}$  microporous membrane to obtain the standard solution. Dilute the standard solution with methanol serially by factors of 2, 4, 8, 16, 32, 64, and 128 to obtain standard solutions at different concentrations. Analyze these solutions using liquid chromatography and record retention times and peak areas. Analyze the linear relationship between peak area and concentration.

### 4.3.5 Chromatographic Conditions

Separation was performed using a Waters CORTECS UPLC T3 column ( $\Phi 100 \times 2.1$  mm, 1.6  $\mu\text{m}$ , S/N 186008499). The column temperature was set to 30  $^{\circ}\text{C}$ , flow rate was 0.3 mL/min, and detection wavelength was 203 nm. The injection volume was 2.0  $\mu\text{L}$ . Mobile phase A was pure water, and mobile phase B was acetonitrile. The gradient elution program is shown in Table 1:

**Table 1:** UPLC chromatographic conditions

t (min)	Mobile phase A: Water (%)	Mobile phase B: Acetonitrile (%)
0	93	7
10	58	42
11	57	43
13.8	54.2	45.8
14.8	53.8	46.2
15	53	47
16	50	50
18	47	53
19	44	56
23	43	57
28-33	0	100
38-43	93	7



**Figure 2:** Testing data- load current (amperes)

#### 4.3.6 Content Determination

Inject the prepared test solutions from each group according to the chromatographic conditions in Table 1. Calculate the content of each ginsenoside component using the linear regression equations obtained from the method described in section 4.3.7.

#### 4.3.7 Data Processing

The linearity of the standard solutions was fitted using Origin software. The content of various saponins in the test solutions was calculated according to their corresponding regression equations. Compare the content of various saponins between processed products and raw materials, calculating the increase in rare saponins after steaming.

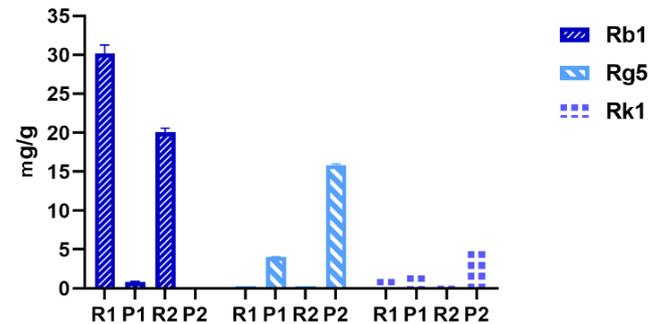
### 4.4 Research Results

Based on the task design in section 4.2, this case study investigated the dynamic changes of saponin components during the steaming process of *Panax ginseng* and *Panax quinquefolius*.

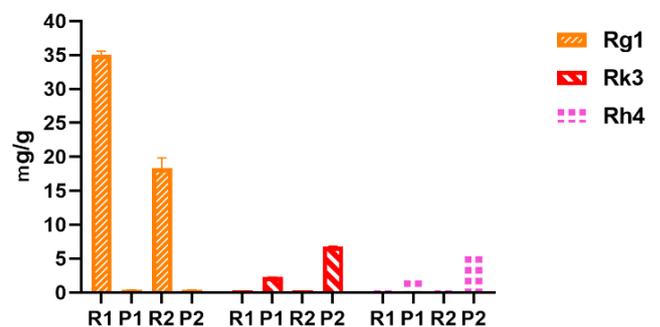
This study selected primary ginsenosides like Rb1, Rg1, and rare ginsenosides Rg5, Rk1, Rk3, Rh4 as key indicators to systematically characterize the dynamic changes of saponins during steaming. Experimental results showed that after processing, the content of primary ginsenosides decreased significantly, while the content of rare ginsenosides showed a gradual upward trend. This pattern indicates that processing is not a simple increase or decrease of single components but a continuous dynamic process where primary ginsenosides gradually transform into rare ginsenosides through hydrolysis, dehydration, and other reactions, reflecting the systematic and directional nature of component transformation during processing.

The mechanism underlying this dynamic pattern mainly stems from the hydrolysis of saponin glycosyl groups under thermal conditions and subsequent structural transformations. Under high temperature and pressure, primary ginsenosides first lose some glycosyl groups, converting to secondary ginsenosides, which then undergo competitive reactions like dehydration and cyclization to generate rare ginsenosides with different structures, such as Rk1 and Rg5. Experimental data indicate that during the processing of both ginseng species, Rg5 and Rh4 (both products of 1,4-elimination reactions) were the predominant products, further confirming the existence of competitive reaction mechanisms among the transformation pathways. Students gained insights from the experiment: in production, it is necessary to precisely control processing

parameters based on the different herbal matrices to directionally obtain target rare ginsenosides. This discovery provides a scientific basis and practical guidance for developing processed products with specific characteristics and efficacy.



**Figure 3:** Changes in the content of PPD-type ginsenosides. Notes: (R1: Ginseng; P1: Prepared ginseng products; R2: American ginseng; P2: Prepared American ginseng products).



**Figure 4:** Changes in the content of PPT-type ginsenosides. Notes: (R1: Ginseng; P1: Prepared ginseng products; R2: American ginseng; P2: Prepared American ginseng products).

### 4.5 Teaching Reflection and Discussion

This case study, using the micro-project “Comparison of the Effects of Steaming Processing on the Composition of Rare Ginsenosides in *Panax ginseng* and *Panax quinquefolius*” as a vehicle, successfully transformed High-Performance Liquid Chromatography (HPLC/UPLC) technology into a cognitive tool for students to actively construct a systematic material perspective of natural medicines.

At the level of technology integration and cognitive deepening, this case achieved a fusion of “technical operation” and “scientific thinking.” Students no longer mechanically executed the “injection-detection-recording” process. Instead, to answer the questions about component dynamics in Task 1, they actively employed HPLC technology as a tool to observe the material world. They needed to independently select primary and rare ginsenosides as key indicators and interpret the chemical significance and dynamic relationships of each peak in the chromatogram. When students deduced the pattern of “decrease in primary ginsenosides, increase in rare ginsenosides” from the data and further explored the underlying competitive reaction mechanisms like hydrolysis and dehydration (Task 2), HPLC technology became a bridge connecting microscopic molecular changes with macroscopic processing techniques, effectively promoting the formation of students’ “dynamic view” and “relational view.”

At the level of systems thinking and transfer application, the comparative task designed in this case (Task 3) was key to

stimulating students' systems thinking. By analyzing the experimental phenomenon where different predominant rare ginsenosides were produced from *Panax ginseng* and *Panax quinquefolius* under the same processing conditions, students gained a deeper understanding of the complexity of the material basis of natural medicines. They realized that processing transformations are not single chemical reactions; their pathways and products are significantly influenced by the inherent primary saponin composition (intrinsic material basis) of the herb itself. This cognitive advancement helped students break free from simple linear thinking and initially establish a systematic view of personalized process design based on herbal/processed product characteristics, which has significant enlightening significance for their future work in Chinese medicine research and production.

This teaching practice also revealed some areas for improvement. Firstly, time and resource limitations remain major challenges for fully open inquiry. The complex sample preparation steps and limited instrument access time reduce students' opportunities for multiple rounds of optimization and data analysis. Secondly, due to insufficient class time, students showed deficiencies in data integration and deep analysis capabilities when processing chromatographic data, for example, facing difficulties in constructing "content-time" curves and performing kinetic analysis.

## 5. Conclusion

Traditional Natural Medicinal Chemistry experiment courses have long suffered from the drawbacks of emphasizing theory over practice and focusing on results while neglecting processes. This teaching model struggles to meet the requirements of cultivating modern pharmaceutical talents. Based on constructivist learning theory, this study proposes focusing on cultivating students' ability to autonomously construct knowledge systems during the experimental process. Regarding specific teaching model innovation, the research takes "chromatography-spectroscopy hyphenated techniques" as the main teaching axis and designs a "phased open" inquiry-based experimental program. Through the three-dimensional teaching design framework of "technique application - context creation - thinking training," the previously fragmented confirmatory experiments are transformed into systematic cognitive tools, achieving a shift in experimental teaching from passive reception to active inquiry. This new experimental teaching model, through the organic integration of advanced techniques and the design concept of phased inquiry, not only effectively enhances students' practical abilities but also promotes their autonomous construction of the knowledge system concerning the material basis of natural medicines, providing a feasible solution to improve the lack of practicality and inquiry in traditional courses.

The case study on teaching reform reflects that this program effectively addresses several difficulties in the learning process, particularly students' weak cognition of the relationship between the holistic effect of drugs and molecular structures, knowledge fragmentation, and insufficient holistic thinking ability. Through open research topics like the comparison of *ginseng* and *American ginseng* processing techniques, students not only mastered the application of

advanced analytical techniques but also gradually developed a three-dimensional cognitive model of "correlation-dynamics-holism" regarding the active components of natural medicines during data processing, pattern discovery, and mechanism exploration. This development reflects an important transformation in learners' thinking patterns from passive recipients of knowledge to active constructors of theory.

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