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Development and validation of a High-Performance Liquid Chromatography method for quantification of total oleanolic acid in *Achyranthes aspera* L.

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ABSTRACT

Background: *Achyranthes aspera* L. is a medicinal plant commonly found in Vietnam, traditionally used for its cooling properties and therapeutic effects on the liver and kidneys, including relieving constipation, reducing blood pressure, and treating hepatitis and polyarthritis. The major bioactive constituents include saponins, alkaloids, flavonoids, and oleanolic acid - a triterpenoid considered a potential marker compound for quality control. **Objective:** This study aimed to establish and validate a reliable analytical procedure for quantifying total oleanolic acid in *A. aspera* to enhance the standardization of its quality assessment. **Method:** Chromatographic separation was achieved on a Shim-pack GIST C18 column (250 × 4.6 mm, 5 μm) using a Shimadzu LC-2030C 3D Plus system. An isocratic mobile phase of acetonitrile and 0.1% phosphoric acid (80:20, v/v) was employed, and detection was performed at 190 nm. The column temperature was maintained at 40°C, with an injection volume of 20 μL and a total run time of 20 minutes. **Results:** The method exhibited excellent linearity over 0.51 - 147.4 μg/mL ($R^2 = 0.9995$). Accuracy, confirmed by recovery tests, ranged from 97.03% to 101.22%, and precision was confirmed with a relative standard deviation (RSD) of 3.3%, demonstrating the method's reliability and suitability for the quantification of oleanolic acid in *Achyranthes aspera*. **Conclusion:** the validated HPLC-DAD method provides a precise, sensitive, and reproducible tool for the quality control and standardization of *Achyranthes aspera*, facilitating its application in research and the development of standardized herbal products.

Keywords: *Achyranthes aspera*, Oleanolic Acid, HPLC-DAD, quantitative analysis, validation

1. INTRODUCTION

Achyranthes aspera L., commonly known as “ngũu tất nam” or “cỏ xước” (CX), is a plant species belonging to the family Amaranthaceae, which is widely distributed throughout various regions of Vietnam. This medicinal herb has traditionally been used to treat conditions such as fever due to common cold or malaria, dysuria, painful urination, irregular menstruation, and uterine blood stasis. With its blood-activating and musculoskeletal-strengthening properties, CX is a common ingredient in numerous traditional formulations that tonify the liver and kidneys and alleviate chronic musculoskeletal disorders [1].

The major chemical constituents of CX include saponins, alkaloids, flavonoids, and oleanolic acid (OA) (Figure 1) - a triterpenoid compound regarded as a potential marker for quality control of this medicinal material [2]. However, the Vietnamese Pharmacopoeia V currently lacks a monograph specifying the quantitative determination of oleanolic acid in CX [3]. Therefore, this study was conducted to develop and validate a quantitative method for determining total oleanolic acid using HPLC-DAD, aiming to enhance the standardization of CX quality control. The successful establishment of

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this analytical procedure not only provides a precise, rapid, and reliable quantification method but also lays the foundation for further research on

formulation development and modernization of CX-based medicinal applications in accordance with international standards.

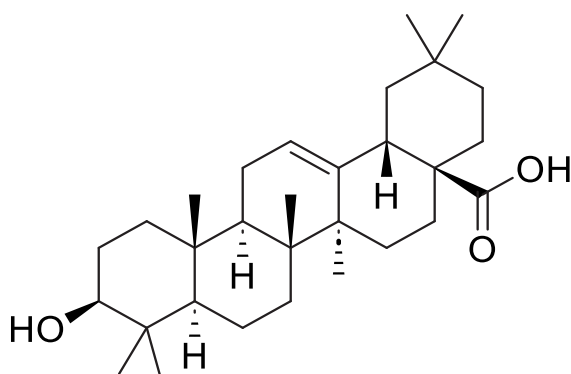


Figure 1. Chemical structures of oleanolic acid used for quantitative analysis in CX

2. MATERIALS AND METHODOLOGY

2.1. Materials

CX used in this study met the quality standards of the Vietnamese Pharmacopoeia V and was supplied by the Ho Chi Minh City Hospital of Traditional Medicine. The botanical identity of the sample was confirmed through DNA barcoding at the University of Science, Vietnam National University, Ho Chi Minh City. After preliminary processing (moisture content below 12%, pulverization, and sieving through a 350 μm mesh), the powdered sample was stored in sealed bags under dry, well-ventilated conditions, protected from direct light and moisture.

2.2. Chemicals and equipment

2.2.1. Chemicals

Oleanolic acid reference standard (ChemFaces, purity 98.0%).

Ethanol (Merck, $\geq 99.9\%$), phosphoric acid (Merck, $\geq 85.0\%$), hydrochloric acid (Gtech, 36-38%), ethyl acetate (Fisher, $\geq 99.8\%$), methanol (Merck, $\geq 99.8\%$), acetonitrile (Merck, $\geq 99.0\%$), chloroform (Merck, $\geq 99.8\%$), HPLC-grade acetonitrile (Fisher, $\geq 99.0\%$), hexane (Xilong, 95 - 98%), single and double distilled water.

2.2.2. Equipments

Chromatographic analyses were performed using a Shimadzu LC-2030C 3D Plus HPLC system equipped with a Shim-pack GIST C18 analytical column (250 \times 4.6 mm, 5 μm) and a Shim-pack GIST C18 guard column (10 \times 4.0 mm, 5 μm). Additional laboratory equipment included a

Sonorex RK-1028H ultrasonic bath, a Memmert WB-14 water bath, analytical balances with 4- and 5-decimal precision, a CN-15-LC UV lamp (254/365 nm), a Gallenkamp vacuum oven, micropipettes covering 10 - 100 μL , 20 - 200 μL , and 100 - 1,000 μL ranges, as well as standard laboratory glassware.

2.3. Methodology

2.3.1. Optimization of the HPLC mobile phase program for simultaneous quantification of OA in CX

The quantification of OA was performed using an HPLC-DAD system (Shimadzu LC-2,030C 3D Plus) with a Shim-pack GIST C18 column (250 \times 4.6 mm; 5 μm) and a C18 guard column (10 \times 4.0 mm; 5 μm). The column temperature was maintained at 40°C, the detection wavelength at 190 nm, and the injection volume at 20 μL .

2.3.2. Optimization of the extraction procedure

According to the Hong Kong Chinese Materia Medica Standards, these preliminary experiments were performed to optimize extracting procedure [4].

Solvent selection

Approximately 1.0 g of powdered CX was placed into a 15 mL centrifuge tube and extracted with 10 mL of solvent (water, 30% EtOH, 50% EtOH, 70% EtOH, or absolute EtOH) using ultrasonication for 20 minutes. The extract was centrifuged at 3,000 rpm for 1 minute, supernatants were collected and transferred to a 10 mL volumetric flask, and adjusted to volume with solvent. After evaporation, the residue was hydrolyzed with 10 mL of 6 M HCl at 80°C for 45 minutes. The

hydrolysate was cooled and extracted with 20 mL ethyl acetate using vortex mixing for 1 minute. The organic layer was collected, evaporated to dryness, and reconstituted in 5 mL of ethanol. The solution was filtered through a 0.45 μm membrane filter and analyzed by HPLC. OA content obtained from different extraction solvents was compared to determine the optimal solvent.

Sample-to-Solvent Ratio selection

Using the optimal solvent obtained above, different material-to-solvent ratios (w/v) were investigated following the same extraction, hydrolysis, and organic extraction steps. The oleanolic acid content was compared among ratios to determine the most efficient extraction proportion.

Extraction time optimization

About 1.0 g of the powdered sample was extracted with 10 mL of the optimized solvent by ultrasonication for 10, 20, or 30 minutes. After extraction, the samples were centrifuged, evaporated, hydrolyzed, and analyzed by HPLC. OA contents were compared to select the optimal extraction duration.

Extract-repetitions selecting

Sequential extractions, consisting of one, two, and three cycles, were conducted under the optimized conditions of solvent type, solvent-to-sample ratio, and extraction time. The resulting extracts were processed according to the described procedure and subsequently analyzed by HPLC. The oleanolic acid (OA) yields obtained from each extraction cycle were compared to determine the optimal number of sequential extractions required.

HCl concentration selection for hydrolysis

Samples were hydrolyzed using HCl solutions of 1 M, 2 M, 4 M, 6 M, and 8 M after extraction under optimized conditions. The hydrolysates were extracted with ethyl acetate, evaporated, dissolved in ethanol, and analyzed by HPLC. The optimal HCl concentration was determined based on OA recovery.

Optimization of hydrolysis conditions

Hydrolysis was performed using 6 M HCl at different temperatures (70, 80, and 90°C) and durations (30, 45, and 60 minutes). After hydrolysis, samples were extracted with 20 mL ethyl acetate, evaporated, dissolved in 5 mL

ethanol, and analyzed by HPLC. The effects of temperature and time were compared to identify optimal hydrolysis conditions.

Effect of organic solvent for liquid-liquid extraction

Under optimized extraction and hydrolysis conditions, OA was extracted from the aqueous phase using various organic solvents—ethyl acetate, chloroform, and hexane. After evaporation and reconstitution in ethanol, the extracts were analyzed by HPLC. The solvent yielding the highest OA content was selected as optimal.

Reconstitution solvent selection

The dried extracts obtained from ethyl acetate extraction were reconstituted in methanol, ethanol, or acetonitrile (5 mL each) and analyzed by HPLC. The solvent providing the best resolution and recovery was selected for subsequent quantification.

2.3.3. Validation of the simultaneous quantification method for OA by HPLC-DAD

The developed HPLC-DAD method for quantifying OA in CX was validated according to ICH guidelines, evaluating system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) [5].

3. RESULTS

3.1. Optimization of HPLC mobile phase program for OA quantification in CX

The mobile phase consisted of acetonitrile and 0.1% phosphoric acid in an isocratic elution mode at a ratio of 80:20 (v/v), with a flow rate of 1.0 mL/min and a total run time of 20 minutes. Under these chromatographic conditions, the OA peak was well resolved with good symmetry and reproducibility, confirming the suitability of the developed HPLC program for subsequent quantitative analysis.

3.2. Extracting procedure optimization

3.2.1. Solvent selection

The influence of extraction solvents on the extraction efficiency of OA from CX was investigated, and the results are presented in Figure 2. Among the tested solvents (water, 30%, 50%, 70%, and 100% ethanol), 50% ethanol yielded the highest OA content. In contrast,

extraction with absolute ethanol (100%) resulted in a significantly lower response, with the OA peak falling below the detection limit.

3.2.2. Investigation of the ratio between sample mass and solvent volume

The OA content in CX was quantified using the HPLC-DAD method at five different ratios (1/5, 1/10, 1/20, and 1/30, w/v) between sample weight

and solvent volume (50% EtOH), as shown in Figure 3.

As shown in Figure 3, the OA content obtained from CX varied across four extraction ratios, with the highest yield observed at a material-to-solvent ratio of 1:10 (w/v). Therefore, this ratio was selected for sample preparation in subsequent experiments.

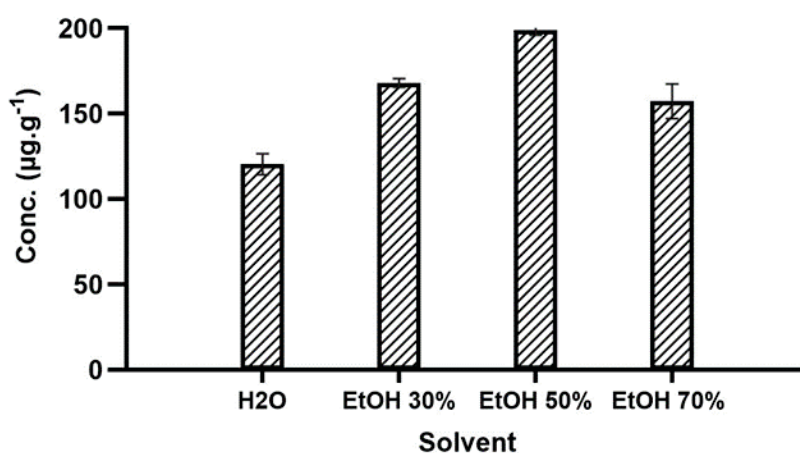


Figure 2. Extraction efficiencies of OA in CX using various solvent systems

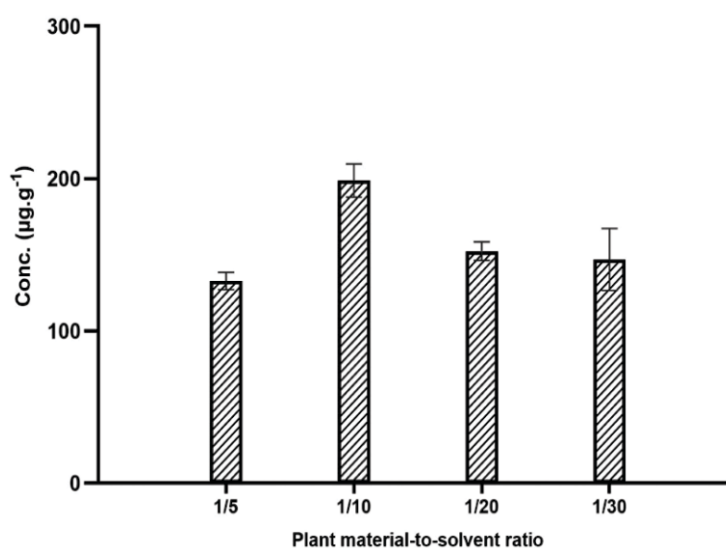


Figure 3. Effect of the sample-to-solvent ratio on the extraction efficiency of OA in CX. Ethanol:water (1:1, v/v) was used as the extraction solvent

3.2.3. Investigation of extraction time

The OA content in CX extracts was determined using the HPLC-DAD method after ultrasonic extraction for 10, 20, and 30 minutes. The results are presented in Figure 4.

The OA content increased significantly when the extraction time was extended from 10 to 20 minutes, indicating efficient solute transfer.

However, further prolonging the extraction to 30 minutes did not enhance the yield and even showed a slight decrease.

3.2.4. Investigation of the number of extraction cycles

The OA content obtained from one, two, and three successive extractions was evaluated using the HPLC-DAD method. The results are presented in Figure 5.

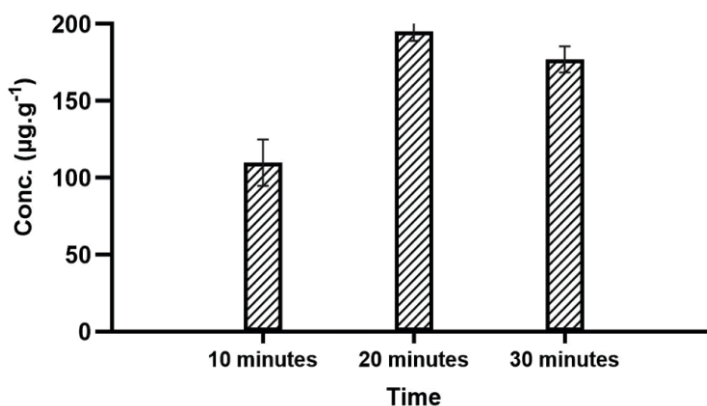


Figure 4. Effect of extraction time on the yields of OA in CX using ultrasonic-assisted extraction with ethanol:water (1:1, v/v)

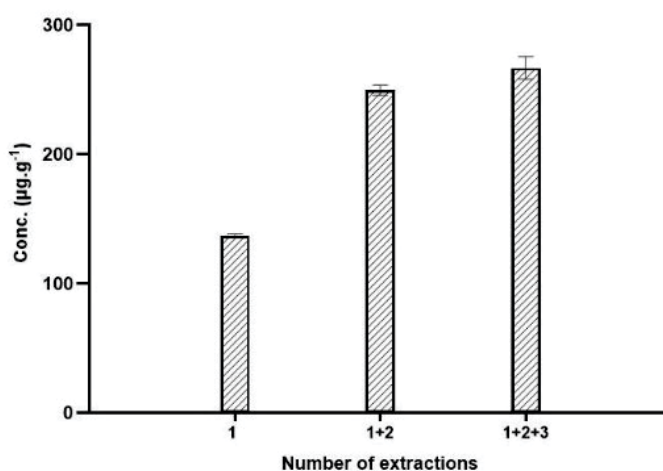


Figure 5. Effect of the number of extraction cycles on the yields of OA in CX using ethanol:water (1:1, v/v) and ultrasonic-assisted extraction

The highest OA content was obtained in the first extraction, indicating that the majority of the analyte was recovered initially. The second extraction still yielded a significant amount, suggesting that some OA remained in the plant matrix. However, the third extraction resulted in a marked decrease in content. Therefore, two

successive extractions were selected as optimal.

3.2.5. Investigation of the HCl concentration for hydrolysis

The OA content obtained at different HCl concentrations was determined using the HPLC-PDA method, and the results are presented in Figure 6.

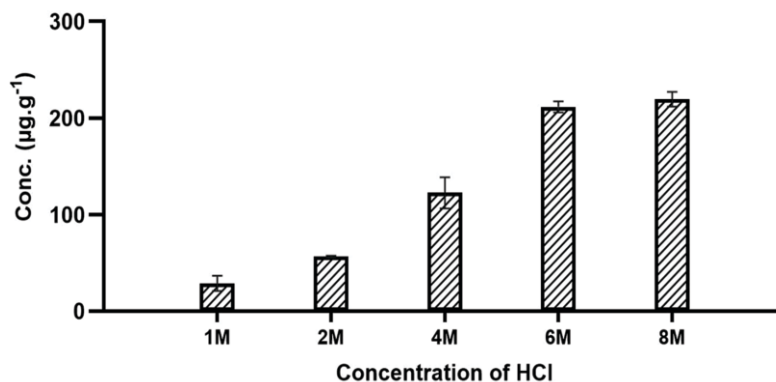


Figure 6. Effect of HCl concentration on the yields of OA in CX using ethanol:water (1:1, v/v) and ultrasonic-assisted extraction

3.2.6. Investigation of hydrolysis conditions

The OA content obtained under different hydrolysis conditions, as determined by HPLC-DAD, is presented in Figure 7.

The results indicate that hydrolysis at 80°C for 45 minutes was determined to be optimal, ensuring complete release of OA while minimizing degradation.

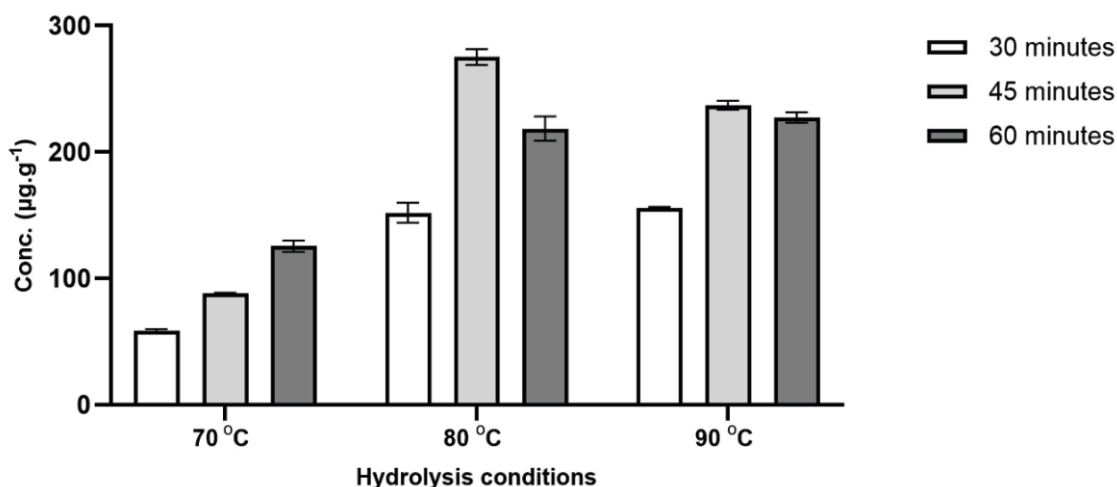


Figure 7. Effect of hydrolysis conditions on the OA content in CX

3.2.7. Investigation of solvents for OA extraction from the aqueous phase

The OA content obtained with different solvents was quantified by HPLC-DAD, and the results are presented in Figure 8.

Based on its chemical structure, OA - a pentacyclic triterpenoid bearing hydroxyl and carboxyl groups - exhibits intermediate polarity. Solvent screening from the aqueous phase indicated that a medium-polarity solvent, such as ethyl acetate, provided superior extraction efficiency compared to less

polar solvents, such as chloroform, or nonpolar solvents, such as hexane. This outcome reflects polarity matching between the solvent and OA, which determines solubility and extraction performance. Accordingly, ethyl acetate was selected as the optimal solvent for this step of the quantification procedure.

3.2.8. Investigation of solvents for sample reconstitution

The OA content after reconstitution in different solvents was quantified by HPLC-DAD, and the results are shown in Figure 9.

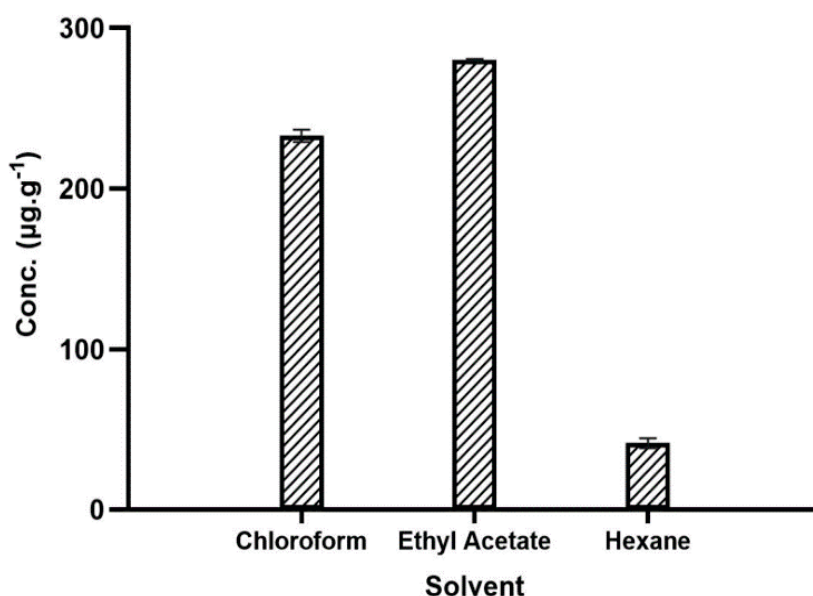


Figure 8. Effect of different organic solvents on the extraction of OA in CX from the aqueous phase

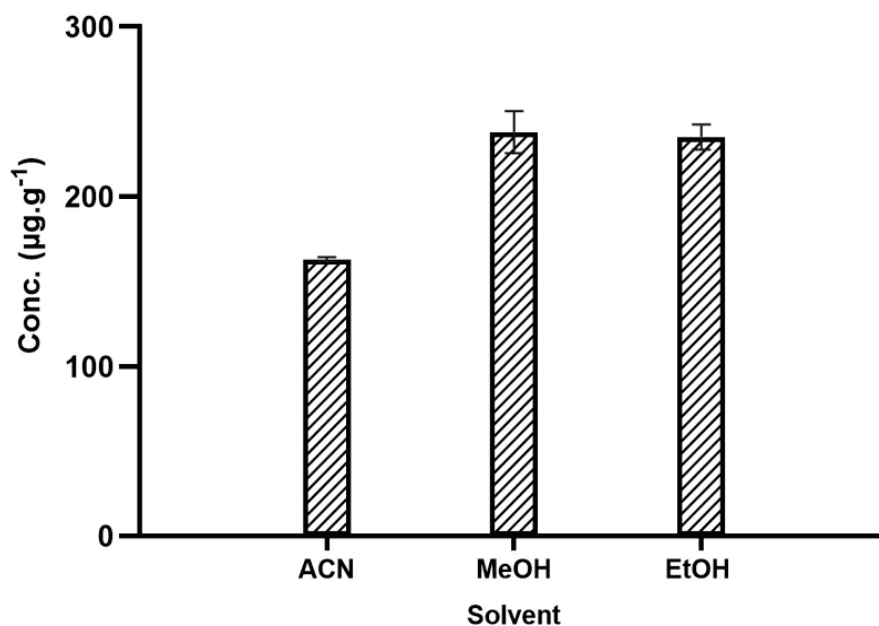


Figure 9. Effect of reconstitution solvent on oleanolic acid solubility

Among the three solvents tested, methanol and ethanol yielded significantly higher OA concentrations than acetonitrile. Although methanol provided the highest solubility, ethanol was selected as the optimal reconstitution solvent due to its lower toxicity, environmental friendliness, good compatibility with chromatographic systems, and comparable solubility. This choice balances extraction efficiency with safety and sustainability considerations in analytical practice.

3.3. Validation of the simultaneous quantification method for OA in CX by HPLC-DAD

3.3.1. System suitability

The system suitability was evaluated by injecting the OA standard solution (100 mg·L⁻¹) seven times. The resolution (R_s) values were consistently greater than 1.5, the tailing factors ranged from 0.5 to 1.5, and the theoretical plate numbers exceeded 2000. These results demonstrate that the HPLC-DAD method for quantifying OA in CX meets the system suitability criteria in accordance with ICH guidelines.

Table 1. System suitability results for the simultaneous quantification of OA in CX

No.	t_R (min)	S (mAU.s)	Tailing F.	NTP (USP)
1	12.838	2,313,319	0.998	13.770
2	12.847	2,325,897	0.999	13.746
3	12.842	2,307,715	0.999	13.821
4	12.832	2,313,494	0.999	13.763
5	12.834	2,309,569	0.999	13.774
6	12.825	2,297,549	1.000	13.784
7	12.843	2,303,082	1.000	13.797
Average	12.837	2,310,089		
RSD (%)	0.06	0.4		

3.3.2. Specificity

As shown in Figure 10, the chromatogram of the blank sample (methanol) exhibited no peaks at the retention time corresponding to OA in the standard solution. The sample chromatogram displayed a peak with a retention time matching that of the OA

standard. Furthermore, spiking the sample with the standard resulted in an increase in both peak height and area. All peaks met the criteria for peak purity assessment, which is shown in Figure 11. These results demonstrate that the HPLC-DAD method for quantifying OA in CX is specific.

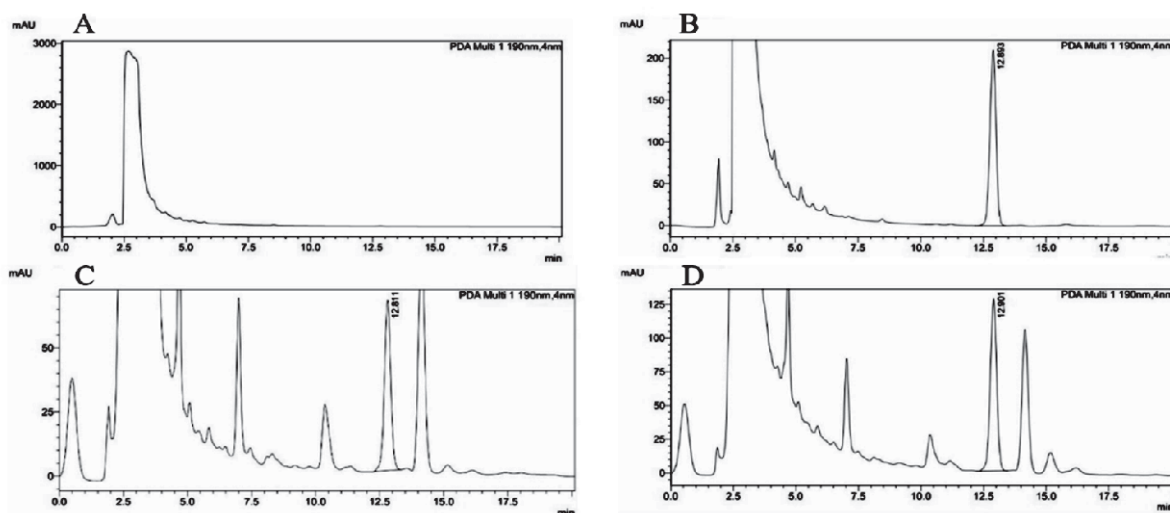


Figure 10. HPLC-DAD chromatograms of blank (A), standard mixture (B), CX sample extract (C), and spiked CX sample extract (D) for the simultaneous quantification of OA in CX

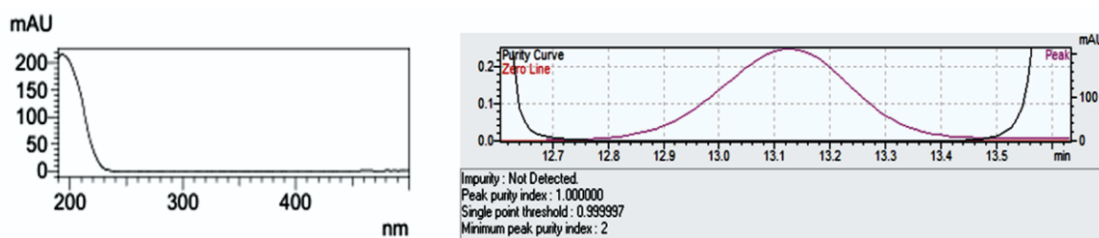


Figure 11. UV spectra and peak purity analysis of OA in CX sample using HPLC-DAD

3.3.3. Linearity

The linearity of the OA quantification method was evaluated, and the results are presented in Table 2 and Figure 12. The calibration curve was expressed as $y = 23627x - 6487.3$ with excellent linearity ($R^2 = 0.9994$). The intercept contributed only 0.28% of the response at $98 \text{ mg}\cdot\text{L}^{-1}$, confirming the suitability of the method for accurate OA quantification in CX.

Table 2. Calibration equations, linear ranges, and correlation coefficients (R^2) for the OA in CX determined by HPLC-DAD

Concentration ($\text{mg}\cdot\text{L}^{-1}$)	S (mAU.s)
2.60	54,243
4.90	101,359
9.80	207,211
14.70	385,752
19.60	469,855
29.40	699,795
49.00	1,096,390
98.00	2,310,089
147.44	3,487,472
Calibration curve	$y = 23,620x - 5,831.6$
R^2	0.9994
%Intercept	0.25%

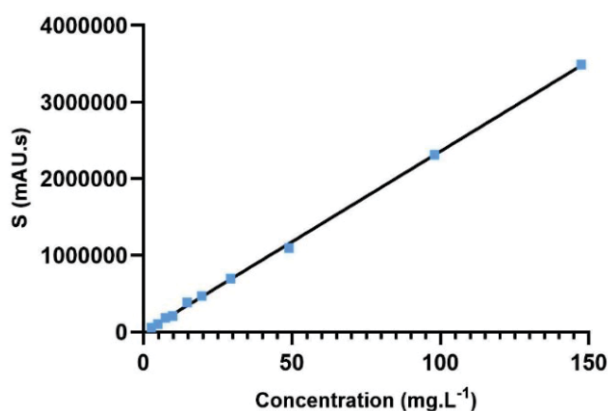


Figure 12. The linear relationship between oleanolic acid concentration and the corresponding HPLC-DAD peak area was established using standard solutions

3.3.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was determined by analyzing OA standard solutions at progressively lower concentrations until the analyte signal was no longer reliably detectable. The standard was initially prepared at $147.44 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$ and serially diluted. At a concentration of $0.51 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$, the signal-to-noise ratio (S/N) was approximately 3, corresponding to the LOD of the method. The LOQ was subsequently

calculated using the formula $LOQ = 10\sigma/S$, yielding a value of $1.55 \mu\text{g}\cdot\text{mL}^{-1}$. These results indicate that

the method is sufficiently sensitive for the detection and quantification of OA in CX.

Table 3. Limits of detection (LOD) and limits of quantification (LOQ) for OA in CX determined by HPLC-DAD (n = 7)

OA ($\mu\text{g}\cdot\text{mL}^{-1}$)	14.7	4.9	2.6	1.1	0.51	0.25
S (mAU.s)	385,752	101,359	54,243	20,199	9936	-

3.3.5. Precision

The results of repeatability and intermediate precision are presented in Tables 4 and 5. The mean content of OA was $240.539 \mu\text{g}\cdot\text{g}^{-1}$, with a relative standard deviation (RSD) of less than 5.3%, which complies with AOAC, Appendix F [7].

Table 4. Repeatability of OA in CX determined by HPLC-DAD

No. (n = 7)	OA concentration ($\mu\text{g}\cdot\text{g}^{-1}$)
Average	239.660
RSD (%)	3.2

Table 5. Reproducibility of OA in CX determined by HPLC-DAD

No. (n = 7)	OA concentration ($\mu\text{g}\cdot\text{g}^{-1}$)		Inter-day precision statistical results
	Day 1	Day 2	
Average	239.660	241.423	
RSD (%)	3.2	3.4	

3.3.6. Accuracy

The accuracy of the OA quantification method in CX was evaluated by the recovery test, and the results are presented in Table 6. The initial OA content in the sample was $240.539 \mu\text{g}\cdot\text{g}^{-1}$. Standard addition was performed at three levels

(80%, 100%, and 120%) to assess recovery. The recovery rates of OA ranged from 97.033% to 101.217%, which are in accordance with the AOAC Appendix F guidelines, demonstrating that the method is accurate and reliable for quantitative analysis.

Table 6. Accuracy (recovery) of OA in CX determined by HPLC-DAD

No.	Spiked standard level (%)	Added Standard (μg)	Recovered Standard (μg)	Recovery (%)
Acid oleanolic				
1	80	199.920	445.337	102.440
2			446.449	102.996
3			436.891	98.215
Average				101.217
RSD (%)				2.58
4	100	239.904	478.702	96.957
5			483.395	98.913
6			474.561	95.231
Average				97.033
RSD (%)				1.90
7	120	287.885	534.024	100.014
8			528.023	97.929
9			519.030	94.806
Average				97.583
RSD (%)				2.69

4. Discussion

OA is both a bioactive compound and a chemical

marker in CX. Previous studies have quantified OA in other species, such as *Gentiana olivieri*

flowers, using HPLC [8], and OA is recognized as a marker in various nutraceutical products [9]. Commercially available reference standards are in the aglycone form, making OA a practical and relevant marker for quality control of CX. In herbal materials, OA occurs as both aglycone and glycoside forms; in this study, hydrolysis was applied to convert glycosides into the aglycone form to accurately reflect total OA content. This approach allows the method to be applied to materials or formulations with low OA content, offering broader applicability than previously reported methods [8].

Due to OA's low UV absorption, 190 nm was selected as the detection wavelength to enhance sensitivity. While β -ecdysterone has been quantified in CX [2], to date, OA content has not been reported, making this study the first to provide quantitative data for OA in *Panax vietnamensis var. fuscidiscus*. Earlier pharmacopoeial data indicated OA content in *Achyranthes bidentata Blume* at approximately 0.1% [4].

Extraction conditions significantly influenced OA yield. OA in CX primarily exists as triterpenoid saponins. Solvents of intermediate polarity, such as 50% ethanol, efficiently extracted both polar sugar moieties and nonpolar aglycones. Highly polar solvents (water or 30% ethanol) resulted in lower extraction efficiency for OA, whereas high ethanol concentrations (70%) reduced the solubility of polar saponins. Therefore, 50% ethanol was selected as the optimal solvent, aligning with pharmacopoeial recommendations and green chemistry principles. The optimal extraction time was 20 minutes; longer extraction led to possible reverse diffusion or slight thermal degradation of saponins.

Hydrolysis with HCl was employed to release OA from glycosides. OA content increased with HCl concentration from 1 M to 8 M, reflecting enhanced cleavage of glycosidic bonds under stronger acidic conditions. A concentration of 6 M HCl was selected to balance hydrolysis efficiency, analyte stability, and operational safety. Hydrolysis efficiency was also temperature- and time-dependent. Increasing the temperature from 70 °C to 80 °C improved OA

release, while further increase to 90 °C slightly reduced yield due to potential degradation. A reaction time of 45 minutes provided maximal OA recovery.

For reconstitution, ethanol was chosen over methanol or acetonitrile due to its higher solubility for OA, attributed to ethanol's polar hydroxyl groups.

Overall, the optimized method provides reliable quantification of total OA in CX, with applicability to both herbal materials and formulations, filling a gap in previous studies and providing a foundation for future quality control and pharmacological investigations.

5. CONCLUSION

In this study, a reliable and robust HPLC-DAD method was successfully developed and validated for the quantification of OA in CX. The initial OA content in the plant material was determined to be approximately 240.539 $\mu\text{g}\cdot\text{g}^{-1}$. Method validation, performed in accordance with ICH and AOAC guidelines, confirmed the method's suitability with respect to system performance, specificity, repeatability, sensitivity, and accuracy. The method demonstrated excellent linearity, with a correlation coefficient (R^2) greater than 0.9994, while the limits of detection (LOD) and quantification (LOQ) were determined to be 0.51 and 1.53 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. Recovery studies at three concentration levels (80%, 100%, and 120%) yielded values ranging from 97.033% to 101.217%, indicating high accuracy and minimal analytical bias. Overall, the developed HPLC-DAD method provides a precise, sensitive, and reproducible approach for the determination of OA in *Achyranthes aspera*, offering a reliable tool for quality control and standardization of this widely used medicinal plant.

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Xây dựng và thẩm định quy trình định lượng tổng acid oleanolic trong cỏ xước bằng phương pháp HPLC-DAD

Dương Hồng Tố Quyên, Nguyễn Thị Thanh Ngân,
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TÓM TẮT

Đặt vấn đề: Cỏ xước (*Achyranthes aspera* L.) là dược liệu phổ biến ở Việt Nam, được sử dụng trong y học cổ truyền với tác dụng thanh nhiệt, hỗ trợ chức năng gan thận, nhuận tràng, hạ huyết áp, điều trị viêm gan và viêm đa khớp. Các hoạt chất chính của dược liệu bao gồm saponin, alkaloid, flavonoid và acid oleanolic - một triterpenoid được xem là hợp chất chỉ thị tiềm năng cho kiểm soát chất lượng. **Mục tiêu:** Nghiên cứu này nhằm xây dựng và thẩm định quy trình phân tích đáng tin cậy để định lượng tổng hàm lượng acid oleanolic trong cỏ xước, góp phần chuẩn hóa đánh giá chất lượng dược liệu. **Phương pháp:** Phân tích sắc ký được thực hiện trên cột Shim-pack GIST C18 (250 × 4.6 mm, 5 μ m) bằng hệ thống Shimadzu LC-2030C 3D Plus. **Kết quả:** Pha động đẳng dòng gồm acetonitril và acid phosphoric 0.1% (80:20, v/v), bước sóng phát hiện 190 nm. Nhiệt độ cột duy trì ở 40°C, thể tích tiêm mẫu 20 μ L, thời gian chạy 20 phút. Phương pháp thể hiện độ tuyến tính tốt trong khoảng 0.51 - 147.4 μ g/mL ($R^2 = 0,9995$). Độ đúng được xác nhận qua phép thử hồi phục với giá trị từ 97.03% đến 101.22%; độ chính xác thể hiện qua RSD (3.3%), chứng tỏ độ tin cậy và khả năng ứng dụng của phương pháp trong định lượng acid oleanolic trong Cỏ xước. **Kết luận:** Phương pháp HPLC-DAD được thẩm định đạt yêu cầu, cho độ chính

xác, độ nhạy và độ lặp lại cao, có thể ứng dụng trong kiểm nghiệm chất lượng và chuẩn hóa dược liệu Cỏ xước, phục vụ nghiên cứu và kiểm nghiệm dược liệu.

Từ khóa: *Cỏ xước, acid oleanolic, HPLC-DAD, phân tích định lượng, thẩm định*

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