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# Determination of Oleanolic Acid Content in *Radix Polysciacis* in Tra Vinh Province

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**Abstract:** *Polyscias fruticosa* (L.) Harms is a traditional medicine, which is planted widely in Tra Vinh province of Vietnam. It is used for nourishing the body, curing asthenia, emaciation, fatigue, poor digestion, poor metabolism, enhance working capacity and brain activity. Oleanolic acid (OA), a marker, is a sapogenin in radix polysciacis. Quantification of OA by high performance liquid chromatography (HPLC) makes an important contribution to the quality assessment of the medicinal plant of *Polyscias fruticosa* (L.) Harms. In order to develop and apply a method to evaluate the content OA in radix polysciacis, planted in Tra Vinh province, many conditions for extracting OA from radix polysciacis were investigated; many HPLC testing conditions were performed find a simple, accurate and fast analytical procedure for the quantification of OA in extract solution of radix polysciacis. As a result, the optimum extraction procedure of OA from the roots of *Polyscias fruticosa* (L.) Harms was found out; a simple, accurate and fast analytical procedure for the quantification of OA in radix polysciacis's extract solution was developed, evaluated and applied to assets the OA content of 05 samples of radix polysciacis collected in different regions of Tra Vinh province. This developed method can control the quality of medicinal materials of *Polyscias fruticosa* (L.) Harms and its preparation effectively.

**Keywords:** *Polyscias fruticosa* (L.) Harms, Oleanolic Acid, HPLC

## 1. Introduction

Radix polysciacis is roots of *Polyscias fruticosa* (L.) Harms (Araliaceae), a traditional medicine that has numerous pharmacological effects like anti-inflammatory, anti-oxidant, anti-depressant and anti-stress. It was used for nourishing the body, curing weakness, emaciation, fatigue, poor digestion, enhancing working capacity, activity brain and also has potential for Parkinson's Disease, social isolation stress-induced brain tissue damage [1-7]. Oleanolic acid (OA) is a sapogenin in the roots and is also a marker of radix polysciacis [8-11]. Quantification of OA by high-performance liquid chromatography (HPLC) makes an important contribution to the quality assessment of radix polysciacis [11-13]. In Tra Vinh, there are many models of growing *Polyscias fruticosa* (L.) Harms that are being implemented, with the output tens tons of radix polysciacis per year. However, at present, there is no research to assess the quality of radix polysciacis grown in Tra Vinh. Therefore, this study was carried out with the goal of building a procedure to quantify OA in *Polyscias fruticosa* (L.) Harms root by HPLC method, applying this process to evaluate the quality of some medicinal samples of radix polysciacis grown in Tra Vinh. Through quantification of OA content in order to make recommendations on expanding the scale of investment in planting, trading and using *Polyscias fruticosa* (L.) Harms.

## 2. Materials and Methods

### *2.1. Materials*

Medicinal material of radix polysciacis (5 years old) was collected in Tra Vinh province in June 2022, They were dried and ground into powder finely (sifted through a 355 sieve).

The reference substance oleanolic acid (content 97.0%), methanol, acetonitrile, double distilled water meet standards for HPLC.

### *2.2. Methods*

#### *2.2.1. Chromatographic Analysis*

The devices used were a Thermo Ultimate 3000 liquid chromatography system (Thermo Scientific, USA), equipped with a vacuum degasser, a quaternary, low-pressure mixing pump, an auto sampler, a thermostat column compartment and a photodiode array detector. The initial proposed chromatographic condition.

Column: Zorbax XDB C8 (250 mm x 4, 6 mm; 5µm) and suitable guard cartridge, Agilent, USA

Detector: PDA

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Mobile phase: mixture of acetonitrile and water

The mobile phase and chromatographic conditions were investigated. Through experimental investigations, choose suitable chromatographic conditions so that the OA peak has purity (according to UV-Vis spectrum), completely removed from impurity peaks (if any) and has an asymmetry in the range of 0.8 – 1.5; minimum resolution is 1.5.

#### *2.2.2. Sample Preparation*

Initial sample preparation: Weigh accurately 5.0 g of radix polysciacis powder, add 50 ml of solvent, ultrasound-assisted extraction in 60 min at 50°C, filter through quantitative filter paper. Filtrated solution was condensed on a water bath at 100°C to dryness. Add 20 ml of 4 M HCl solution to the residue, heat in a water bath for 60 minutes at 90°C to hydrolyze. Transfer the cooled solution to a 100 ml decanter, extract with 20 ml of chloroform (2 times), wash excess acid in the chloroform extract by shaking with 40 ml of water until neutral, concentrate the chloroform extract to residue. Dissolve residue in a 5 ml volumetric with methanol. The samples were filtrated by 0.45 µm membrane prior to HPLC analysis.

Solvent extraction selection: using different solvent: methanol, ethanol 96%, and water to extract OA in radix polysciacis powder as above procedure. Conduct chromatographic analysis these extract solutions and select the solvent for the highest concentration of OA in the extract.

Ultrasonic extraction time selection: use the selected above solvent to investigate the time of ultrasonic extraction for sample preparation: 15 minutes, 30 minutes and 60 minutes. Perform chromatographic analysis of the extracts. Select the extraction time for the highest concentration of OA in the extract.

Extraction times selection: conduct chromatographic analysis of the chloroform extraction of the first time, second time and third time. Select the smallest number of extractions to obtain the maximum amount of OA.

Hydrolysis time selection: conduct chromatographic analysis of the sample that was hydrolyzed with different time: 60 minutes, 120 minutes and 180 minutes. Select the hydrolysis time for the highest OA concentration.

#### *2.2.3. Validation of OA Quantification Procedure in Radix Polysciacis*

The analytical procedure to determine OA in radix polysciacis is proceeded base on ICH harmonised tripartite guideline, validation characteristics are including: system suitability testing, specificity, linearity, precision, accuracy [14].

## 3. Result and Discussion

### *3.1. Chromatographic Analysis*

Investigation of OA quantitative chromatographic conditions is presented in Table 1. UV-Vis spectrum of oleanolic acid and chromatography with different chromatographic conditions was displayed in Figure 1 and Figure 2, respectively.

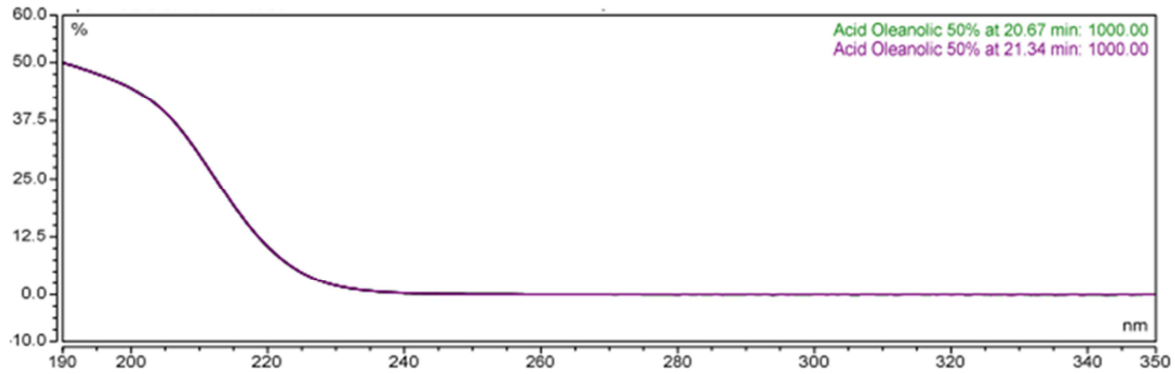


***Figure 1.*** *UV-Vis spectrum of oleanolic acid.*

***Table 1.*** *Quantitative chromatographic conditions of OA.*

| Isocratic Programme | Mobile phase; run time; flow rate (ml/min); column stationary phase |
|---|---|
| I | $CH_3CN$ – $H_2O$ (70: 30); 60 min; F = 1,0; Octylsilane |
| II | $CH_3CN$ – $H_2O$ (70: 30); 60 min; F = 1,0; Octadecylsilane |

| Gradient Programme | Run time, $CH_3CN - H_2O$; flow rate (ml/min); column temperature; column stationary phase |
|---|---|
| III | 0 – 45 min (60: 40), 45,1 – 55 min (90: 10), 55,1 - 65 min (60: 40); F = 1,0; 25°C; C18 |
| IV | 0 – 32 min (67: 33), 32,1 – 45 min (90: 10), 45,1 - 55 min (67: 33), F = 1,0; 25°C; C18 |
| V | 0 – 25 min (67: 33), 25,1 – 35 min (90: 10), 35,1 - 45 min (67: 33), F = 1,2; 25°C; C18 |
| VI | 0 – 25 min (67: 33), 25,1 – 35 min (90: 10), 35,1 - 45 min (67: 33), F = 1,2; 45°C; C18 |

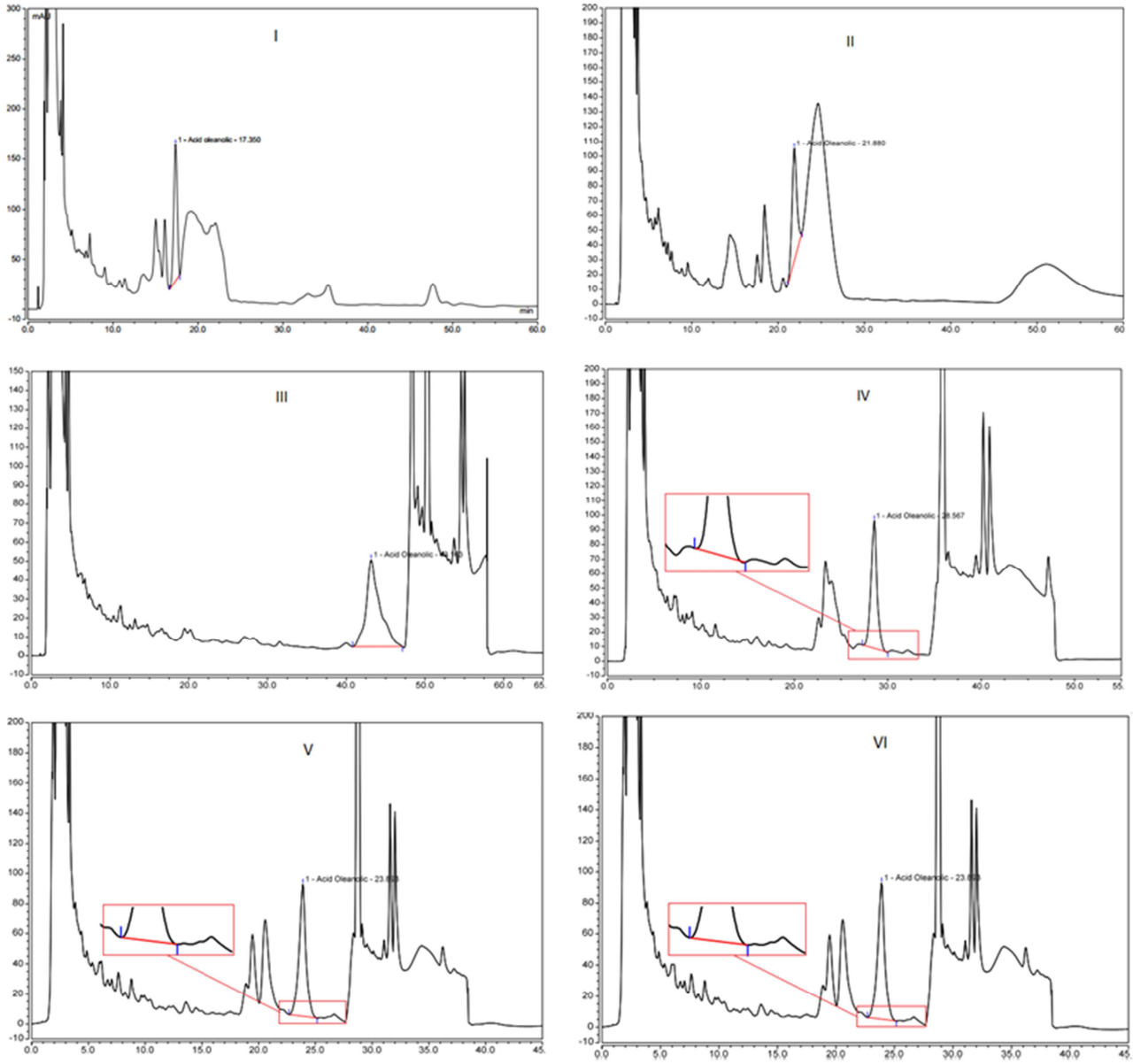


***Figure 2.*** *Sample's chromatography according to different chromatographic conditions.*

The UV-Vis spectrum of OA in the wavelength range 190 - 350 nm has no maximum absorption peak, the absorbance gradually decreases from 190 nm to about 240 nm, so the 203 nm wavelength is chosen as the detection wavelength to ensure the sensitivity of the method. With the isocratic program, the chromatographic parameters OA peak are not satisfactory and the run time is so long. With the gradient program, the program V, VI gives OA peaks that reach the required chromatographic parameters in the shortest time when compared to the others. However, program VI (column temperature is 45°C) did not significantly improve run time and separation when comparing with program V (with column temperature is 25°C – room temperature). The separation ability of the column with the stationary phase C8 and C18 did not differ too significantly in the initial investigation steps, so the column selected was the stationary phase column C18 which was more common. The column temperature was 25°C, which was room temperature, so it was very convenient for the chromatographic process. Finally, the selected chromatographic conditions: Zorbax Eclipse XDB C18 column (250 x 4.6 mm; 5 µm) and Zorbax Eclipse XDB C18 guard cartridge (4.6 x 12.5 mm; 5 µm), flow rate 1, 2 ml/min, column temperature 25°C, detection wavelength 203 nm, sample injection volume 20 µl, mobile phase:

acetonitrile and water with gradient program: 0 – 25 min (67: 33), 25.1 – 35 minutes (90: 10), 35.1 – 45 minutes (67: 33), run time was 45 minutes.

### *3.2. Sample Preparation*

The result of sample preparation testing is displayed in Figure 3.

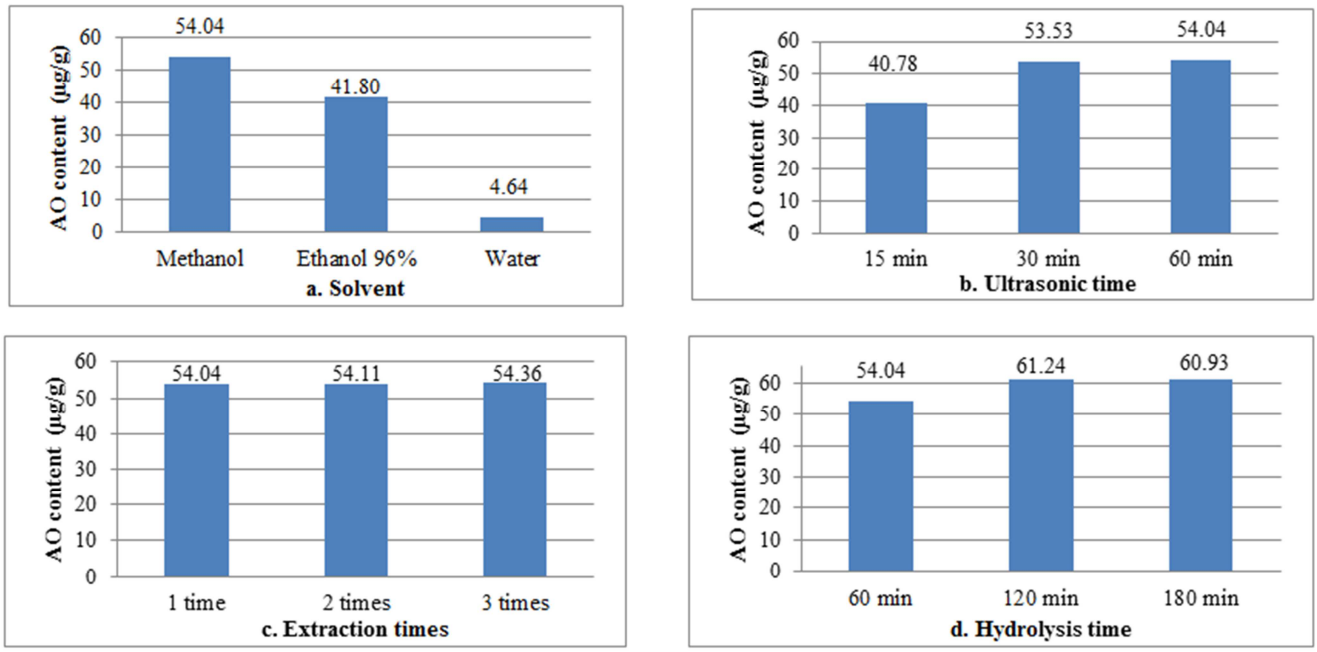


***Figure 3.*** *Result of sample preparation testing.*

HPLC results showed that all 3 solvents, methanol, ethanol 96%, and water could extract OA, but the OA content was gradually increased in the order: water < ethanol 96% < methanol (Figure 3a). Extracted OA content at a time interval of 15 minutes < 30 minutes = 60 minutes (Figure 3b). The content of OA extracted in different time: one time, two times, three times had no statistically significant difference ($p > 0.05$) (Figure 3c). The hydrolyzed OA content was gradually increased as follows: 30 min hydrolysis < 60 min hydrolysis < 120 min hydrolysis = 180 min hydrolysis (Figure 3d).

In conclusion, the chosen conditions for extracting OA in radix polysciacis were: ratio of sample-methanol was 1: 10, extracted once time by ultrasonic method for 30 minutes, then hydrolyzed for 120 minutes. Hydrolyzed solution was continually extracted with chloroform twice.

### *3.3. Validation of OA Quantification Procedure in Radix Polysciacis*

#### *3.3.1. System Suitability Testing*

The results of system suitability testing were performed on standard sample (6 times repeated injection), test sample (6 test samples).

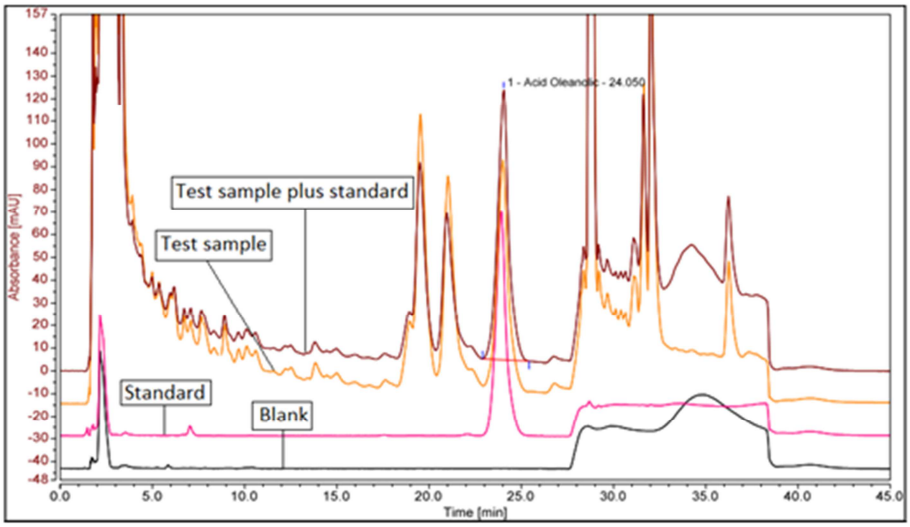


***Figure 4.*** *Overlapping chromatograms of blank, standard, test sample, and standard addition test sample.*

***Table 2.*** *System suitability testing results.*

| | Retention time (min) | Area (mAU. min) | Peak asymmetry | Plate number | Match |
|---|---|---|---|---|---|
| Standard sample | | | | | |
| Average | 23,942 | 64,748 | 1,10 | 10.279 | 1.000 |
| RSD% | 0,07% | 0,71% | 0,8 ≤ As ≤ 1,2 | | |
| Test sample | | | | | |
| Average | 24,012 | 71,661 | 1,06 | 9.044 | 982 |
| RSD% | 0,04% | 2,61%* | 0,8 ≤ As ≤ 1,2 | | |

*: RSD% at the concentration according to Ludwig Huber: ≤ 3.7% [15].

The parameters of retention time, peak area have RSD% ≤ 2%. The asymmetry of peak, plate number and peak purity meet analytical requirements.

### *3.3.2. Specificity*

The results of the specificity test are illustrated in Figure 4.

The chromatogram of the blank sample (methanol solvent) has no peak appearing in the retention time corresponding to the retention time of the OA peak in the standard sample. Chromatogram of the test sample with a peak with a retention time corresponding to the peak OA in the standard sample. When a standard is added to the test sample, the height and area of the peak OA increase. Pic OA on the test solution chromatogram obtained high purity (compatibility factor 982).

### *3.3.3. Linearity*

The results of the correlation between peak area and OA concentration are shown in Figure 5.

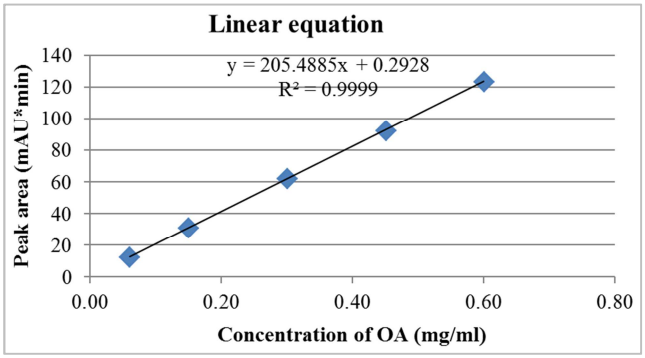


***Figure 5.*** *Correlation between concentration and peak area of OA.*

Statistical processing results show that there is a high linear correlation ($R^2$ = 0.9999) between peak area and OA concentration in the concentration range 0.06 - 0.60 mg/ml.

### *3.3.4. Precision*

*Repeatability*

The repeatability results were carried out on six independent test samples. The average results and RSD% of OA content in the roots were 66.19 µg/g, 2.63% (RSD% at this concentration according to Ludwig Huber: ≤ 3.7% [15]).

*Intermediate Precision*

Intermediate precision was performed in two different dates and personnel. The average results and RSD% of OA content in the roots of analyst 1 and analyst 2 were: 66.19 µg/g, 2.63% and 66.72 µg/g, 2.83%, respectively (RSD% at this concentration according to Ludwig Huber: ≤ 3.7% [15]).

### *3.3.5. Accuracy*

Accuracy was carried out by adding standards to the test sample at 3 levels of 80%, 100% and 120% of the quantitative concentration of OA. Prepare 3 samples for each level. The survey results are correct at each concentration level, the recovery rate is in the range of 98.68 – 103.15% and the RSD% at each concentration level is less than 2.57% (Recovery rate at this concentration level according to Ludwig Huber: 95.0 - 105.0% [15]).

### *3.4. Determination of OA Content in Radix Polysciacis*

Standard sample: OA standard with a concentration of 0.3 mg/ml in methanol.

Test sample: 5.0 g of radix polysciacis powder (sifted through a sieve of 355), add 50 ml of methanol, shake in ultrasonic tank for 30 min at 50°C, filter through filter paper, evaporate the filtrate in a water bath at 100°C. Add 20 ml of 4 M HCl solution to the bite, continue to boil in a water bath for 120 minutes at 90°C to hydrolyze. Extracted with 20 ml of chloroform (2 times), neutralized the chloroform extract by shaking with 40 ml of water (2 times) until neutral, concentrate the chloroform extract to bite. Dissolve in a volumetric flask containing 5 ml of methanol and filter through a 0.45 µm PTFE filter.

HPLC conditions: UHPLC Dionex Ultimate 3000, DAD-3000 RS detector, Zorbax Eclipse XDB C18 column (250 x 4.6 mm; 5 µm) and Zorbax Eclipse XDB C18 guard cartridge (12.5 x 4.6 mm; 5µm), flow rate 1.2 ml/min, column temperature 25°C, detection wavelength of 203 nm, injection volume 20 µl, mobile phase: acetonitrile and water with gradient program: 0 – 25 minutes (67: 33), 25.1 – 35 minutes (90: 10), 35.1– 45 minutes (67: 33), total time 45 minutes.

***Table 3.*** *Results of survey of OA content in 05 samples of radix polysciacis.*

| Sample | Sample weight (g) | Concentration (µg/g) | Concentration (%) |
|---|---|---|---|
| 1 | 5,0013 | 57,32 | 0,0057 |
| 2 | 5,0035 | 66,15 | 0,0066 |
| 3 | 5,0076 | 72,13 | 0,0072 |
| 4 | 5,0034 | 63,71 | 0,0063 |
| 5 | 5,0023 | 55,33 | 0,0055 |
| | | Average 62,93 | 0,0063 |
| | | RSD% 10,8 | 10,8 |

Quantitative results of OA content of 05 samples of radix polysciacis are in the range of 55.33 - 72.13 µg/g, equivalent to 0.0055 - 0.0072%. The results showed that concentration of OA in the roots was quite low, about 0.0063% and there was

not much variation in the OA content of the roots harvested in different regions (RSD% about 10.8%).

## 4. Conclusion

The OA quantification process has been built and validated to meet the requirements of system suitability testing, linearity ($R^2 = 0.9999$), specificity, precision and high accuracy (RSD% intermediate accuracy was 2.64%). The accuracy with the recovery of OA was in the range of 98.68 – 103.15%. The content of OA in medicinal herbs of 05 samples of radix polysciacis harvested from different regions of Tra Vinh province ranged from 55.33 to 72.13 µg/g (0.0055 - 0.0072%).

## Acknowledgements

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