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# An Anion Exchange Monolithic Cartridge for Extraction and Analysis of Oleanolic Acid from *Oldenlandia diffusa*

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An anion exchange monolithic cartridge was synthesized and used for extraction and analysis of oleanolic acid from *Oldenlandia diffusa*. The analysis was performed on HPLC with an eluting solution consisting of methanol-water-acetic acid (88:12:0.5, v/v/v, pH = 5.8) and a $C_{18}$ column. An ultrasonic-assisted process was applied to accelerate the extraction process, which was obviously increasing the extraction efficiency. Under the optimum condition, good linearity was obtained in a range of 50-2000 μg $mL^{-1}$. The intra-day and inter-day were less than 4.8 and 5.7 % and recoveries were ranging from 98.3-104.5 %. This method can be used for the extraction and analysis of drugs from natural plants due to its simplicity and reliability.

**Key Words: *Oldenlandia diffusa*, Oleanolic acid, Extraction, Anion exchange, Monolithic cartridge.**

## INTRODUCTION

*Oldenlandia diffusa* (*O. diffusa*) is a well-known medicinal plant commonly used in China[1,2]. It has been found to possess notable effect on treatment of many types of diseases in clinical application[3,4], such as hepatitis, tonsillitis, sore throat, appendicitis and urethral infection. Recently, this herb has gained increasing attention for further utilization in the treatment of malignant tumours[5,6]. Many studies of *O. diffusa* have demonstrated many bioactive components in the plant, like oleanolic acid and quercetin[1,7].

Oleanolic acid (Fig. 1) is a naturally occurring triterpenoid, widely distributed in food and medicinal plants, with relatively non-toxic and exhibiting antiviral properties[2]. Liquid-liquid extraction is a common extraction technique for natural plants, with the extraction sample generally requiring pretreatment step for further purification prior to HPLC analysis[8,9]. Solid phase extraction with inexpensive and efficient materials has recently been applied and is a viable alternative to conventional sample preparation methods[10,11]. Previous studies have shown that ion exchange material with special functional groups has the potential to be better stationary phases in chromatography, especially as solid phase extraction materials[12,13]. In this study, a solid phase extraction method was developed to extract oleanolic acid from *O. diffusa* using an anion exchange monolithic cartridge.

## EXPERIMENTAL

*O. diffusa* was bought from local market (Incheon, Korea). Oleanolic acid, methacrylic acid (MAA) and glycidyl methacrylate (GMA) were bought from Sigma (St Louis, MO, USA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Fluka (Buchs, Switzerland). Methanol, ethanol, chloroform, acetone, diethylamine and acetic acid were bought from Duksan Pure Chemical Co., Ltd (Ansan, Korea).

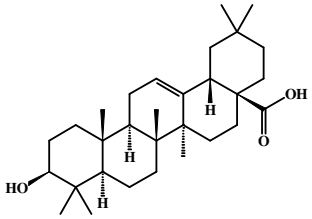


Fig. 1. Chemical structure of oleanolic acid

The chromatography system consisted of Waters 600 s Multi solvent Delivery System, Waters 616 liquid chromatography (Waters Associates, Milford, MA, USA), a Rheodyne injector (20 μL sample loop) and a variable wavelength 2487 UV dual channel detector. Autochro-2000 software (Younglin Co. Ltd., Korea) was used as data acquisition system. The analysis was performed on an OptimaPak $C_{18}$ column (5 μm, 150 mm × 4.6 mm, i.d., RS Tech Corporation, Daejeon, Korea). All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, USA) before injection into the HPLC system.

**Preparation of anion exchange monolithic cartridge[12,13]:** First, a mixture consisting of 0.5 mL methacrylic acid, 2 mL glycidyl methacrylate, 3 mL ethylene glycol dimethacrylate,

3 mL dodecanol, 3 mL cyclohexanol and 0.06 g AIBN was purged with helium gas for 15 min. Then, a stainless-steel cartridge (150 mm × 4.6 mm, i.d.) was filled with the mixture and sealed at the top. After the proceeded at 55 ºC for 24 h, the cartridge was flushed with methanol to remove the interfering matrices. A mixture of diethylamine and tetrahydrofuran (1:1, v/v) was then pumped through the cartridge at 0.1 mL/min in steps at 80 ºC for 24 h. Finally, the cartridge was washed with 0.05 mol/L acetate buffer, followed by deionized water, until the eluent was neutral. The cartridge was cut into pieces of a certain size (20 mm × 4.6 mm, i.d.) for further SPE process.

**Morphology analysis:** The monolithic polymer was grounded and sieved to small particles for analysis. The BET specific surface areas and pore size distributions of the samples were measured through nitrogen adsorption at liquid nitrogen temperature with a Micromeritics ASAP 2020 system (Micromeritics, USA).

**Sample preparation and chromatography:** Stock standard solutions were prepared by dissolving an appropriate amount of the drug in methanol at a final concentration of 3000 μg $mL^{-1}$. A series of mixed standard solutions containing oleanolic acid were prepared at seven different concentration levels from 50-2000 μg $mL^{-1}$. Chromatography analysis was performed on a $C_{18}$ column at ambient temperature. The optimum mobile phase was methanol-water-acetic acid (88:12:0.5, v/v/v, pH = 5.8) at a flow rate of 0.8 mL $min^{-1}$ with the UV wavelength of 215 nm.

**Extraction of *O. diffusa*:** 2.0 g of *O. diffusa* powders were dissolved in 40 mL of five solvents (water, methanol, ethanol, chloroform and acetone). Extraction time (0.25, 0.5, 1 and 2 h) with ultrasonication was also investigated and extraction temperatures were all set at 25 ºC. After stirring, the sample extracts were filtered and the solutions were dried and dissolved into 2 mL methanol. Then, 1 mL of sample was added to the cartridge, followed by 10 mL of water to remove interfering matrices. Finally, the extracts were eluted with 10 mL of methanol and the outflow solutions collected separately. The solutions were filtered and directly injected into the HPLC.

## RESULTS AND DISCUSSION

**Morphological characteristic:** Nitrogen adsorption experiments were performed and the specific surface area (35.62 $m^2$ $g^{-1}$) and pore volume (0.11 $cm^3$ $g^{-1}$) of monolithic polymer were obtained. The average pore diameter was calculated to be 22.6 nm (BJH method).

**Optimization of chromatographic conditions:** Methanol-water system was first tested as the mobile phase and acetic acid was involved as an additive to the eluting solution. The retention time of oleanolic acid on the $C_{18}$ column was greatly affected by the methanol content in the mobile phase and an optimum mobile phase composed of methanol-water-acetic acid (88:12:0.5, v/v/v, pH = 5.8) was obtained with UV detection at 215 nm.

**Optimization of extraction process:** Ultrasonication did not significantly improve the extraction efficiency, but it can greatly reduce the time required[14]. The extraction solvent was optimized by examining five different extractant solvents and the extraction time (0.25, 0.5, 1 and 2 h) was also investigated for optimum yield. Experimental results showed that the extraction yield didn't increase significantly when the extraction time exceeded 0.5 h. Fig. 2 showed the extraction yields of oleanolic acid from *O. diffusa* by five different extractant solvents and indicated a minor difference between chloroform and acetone regarding yields. According to the toxicity of chloroform, the optimum extraction condition (acetone as extraction solvent, 0.5 h and 25 ºC) with ultrasonication was obtained for extraction of oleanolic acid from *O. diffusa*.

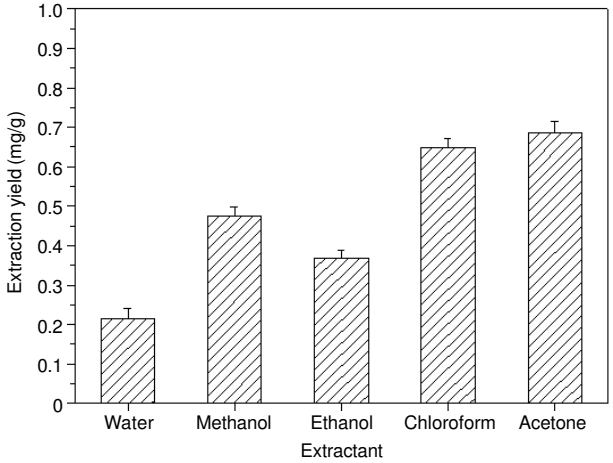


Fig. 2. Extraction yield of oleanolic acid from *O. diffusa* by five different extractant solvents

An anion exchange monolithic cartridge was employed as an SPE column to process the extract samples. The interfering species of extract samples were removed by 10 mL of water and oleanolic acid as target compound was eluted with 10 mL of MeOH and the outflow solution was collected for analysis on a $C_{18}$ column using the optimum mobile phase.

**Method validation:** Good linearity of oleanolic acid was obtained for the analyte throughout the concentration range of 50-2000 μg $mL^{-1}$ with a regression equation of: Y = 105.38 + 16.30 X ($r^2$ = 0.9997). Based on the signal-to-noise ratio of 3 and 10, the limit of detection (LOD) and limit of quantification (LOQ) were 10 and 30 μg $mL^{-1}$ for oleanolic acid, respectively.

Intra- and inter-assay precision were expressed at three concentration levels on five different days (n = 5) while the relative standard deviation (RSD) were less than 4.8 and 5.7 %. The method recovery was tested to investigate the reliability of method by spiking three different concentrations (100, 500 and 1500 μg $mL^{-1}$) of oleanolic acid into the extract samples, with the results shown in Table-1. The mean recoveries for the analyte ranged from 98.3-104.5 % with a RSD less than 4.6 %, indicating a reliable method.

***O. diffusa* extract sample analysis:** The HPLC chromatogram of the acetone extract samples without SPE (a) and with SPE (b) by anion exchange monolithic cartridges is shown in Fig. 3. The results indicate that the SPE monolithic cartridge was effective at eliminating the interfering peaks and had a high recovery of the analyte. Using this established method, the extract yield of oleanolic acid from *O. diffusa* was 0.685 mg $g^{-1}$.

TABLE-1
INTRA-DAY AND INTER-DAY PRECISIONS, ACCURACIES AND RECOVERIES OF OLEANOLIC ACID WITH THREE DIFFERENT CONCENTRATIONS

| | Concentration (μg/mL) | Intra-day Precision RSD (%) | Inter-day Precision RSD (%) | Recovery (%) |
|---|---|---|---|---|
| Oleanolic acid | 100.0 | 3.3 | 4.0 | 98.3 |
| | 500.0 | 4.5 | 5.1 | 104.5 |
| | 1500.0 | 4.8 | 5.7 | 100.9 |

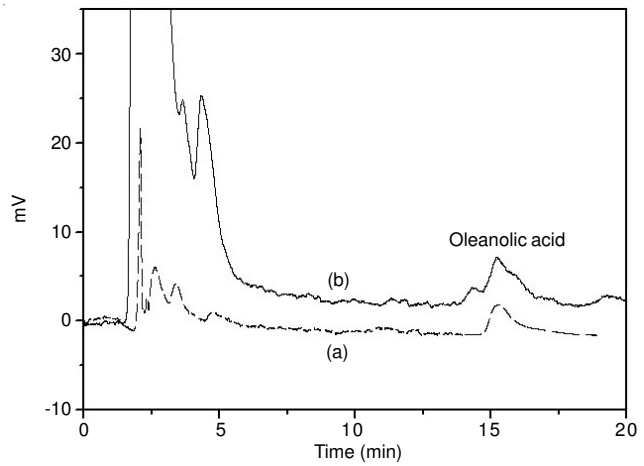


Fig. 3. Chromatogram of oleanolic acid extract sample by acetone with SPE (a) and without SPE (b)

## Conclusion

An anion exchange monolithic cartridge was synthesized as a SPE material for purification of oleanolic acid from *O. diffusa*. An ultrasonic-assisted process applied to accelerate the extraction process, which increased the extraction efficiency. This SPE method had required precision, accuracy and recovery, which demonstrated its reliability for drug assays. The results showed that the recoveries of oleanolic acid from three spiked levels were in the range of 98.3-104.5 %, with an RSD less than 4.6 %. This method can be used for the extraction and analysis of drugs from natural plants given its simplicity and reliability.

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