Preparative Separation of High-Purity Dihydroartemisinic Acid from Artemisinin Production Waste by Combined Chromatography

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In order to make full use of artemisinin production waste and thus to reduce the production cost of artemisinin, we developed an efficient and scalable method to isolate high-purity dihydroartemisinic acid from artemisinin production waste by combining anion-exchange resin with silica-gel column chromatography. The adsorption and desorption characteristics of dihydroartemisinic acid on 10 types of anion-exchange resin were investigated, and the results showed that the 717 anion-exchange resin exhibited the highest capacity of adsorption and desorption to dihydroartemisinic acid. Adsorption isotherms were established for the 717 anion-exchange resin and they fitted well with both Langmuir and Freundlich model. Dynamic adsorption and desorption properties of 717 anion-exchange resin were characterized to optimize the chromatographic conditions. Subsequently, the silica-gel column chromatography was performed and dihydroartemisinic acid with a purity of up to 98% (w/w) was obtained. Finally, the scale-up experiments validated the preparative separation of high-purity dihydroartemisinic acid from industrial waste developed in the present work. This work presented for the first time an isolation of dihydroartemisinic acid with a purity of 98% from Artemisia annua (A. annua) by-product, which adds more value to this crop and has the potential to lower the prices of anti-malarial drugs.

Key words: anion-exchange resin; Artemisia annua L.; artemisinin production waste; dihydroartemisinic acid; silica-gel chromatography

Malaria is one of the world’s most important parasitic diseases, leading to approximately one million deaths, and affects more than 300 million people per year worldwide (WHO, 2016). Because Plasmodium falciparum, the agent of the fatal cerebral malaria, developed drug resistance to quinine, artemisinin (ART) became the only effective and safe drug to combat malaria. In 2016, artemisinin combination therapies (ACT) accounted for 33% of the global demand for antimalarial medicines (1.5 billion treatment courses), with the ACT demand expected to grow to 45% on antimalarial medicines in 2019. That is equivalent to 329 million ACT treatment courses (164.5t of ART) to be needed in 2019. Besides its antimalarial effects, ART and its derivatives were reported to have antitumor activity. Also, ART can increase γ-aminobutyric acid (GABA) receptor signaling and prevent gluconon glucagon secretion by inhibiting α-cell identity and the transcription factor Arx, suggesting its potential application in the treatment of diabetes. Therefore, it is expected that the demand for ART would expand sharply in the near future.

However, ART-based antimalarials are still unaffordable for Africans who need it the most due to the high production costs of ART. Although production of ART in genetically-engineered yeast has been tried to reduce the cost of ART production, only artemisinic acid was obtained and production logistics led Sanofi to sell its plant in Italy. Currently, Artemisia annua L. is still the only source for the commercial ART, which makes this plant be a very important pharmaceutical crop in Asia and Africa. The isolation of ART from A. annua leads to a large amount of ART production waste (APW) generated per year. APW is the oily residue of petrol extract from A. annua leaves after ART was isolated. And the amount of APW keeps increasing since the demand of ART continues to increase. Our previous work reported that dihydroartemisinic acid (DHAA) was present in APW. DHAA (Fig. 1) is the main direct precursor of ART in A. annua, and it is believed that DHAA could be photochemically converted into ART, with dihydroartemisinic acid hydroperoxide as an intermediate, in vitro, and without the participation of any enzyme. The isolation and identification of dihydroartemisinic acid hydroperoxide by Wallaart et al. provided strong evidence of an non-enzymatic conversion of DHAA into ART in vivo. Recently, an efficient method for in vitro conversion of DHAA to ART in large scale was developed using singlet oxygen and another method reported a 39% yield in the conversion of DHAA into ART with potential conversion improved to over 60%. This high-efficient conversion in vitro provides a potential avenue to improve the ef-

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ficiency of ART production through full utilization of DHAA. Although Wallaart et al.\textsuperscript{14} were the first to report the isolation of DHAA from leaves of \textit{A. annua}, their isolation was time-consuming and only suited for isolation at a laboratory scale. In our previous research, we described a simultaneous isolation of ART and its precursors from leaves of \textit{A. annua} by preparative RP-HPLC, but it requires expensive instrumentation.\textsuperscript{19} Kohler et al.\textsuperscript{19} developed the extraction of ART and AA (artemisinic acid) from \textit{A. annua} leaves using supercritical carbon dioxide, but DHAA was not separated in their work. Moreover, all of the reported methods for the isolation of DHAA focused on its extraction and isolation from \textit{A. annua}, but none has attempted to isolate DHAA from APW. Recently, we described an ultrasound-assisted extraction of DHAA from the APW.\textsuperscript{10} However, this method only extracted crude DHAA from APW.

Column chromatography is widely used for separation and purification of bioactive compounds from plant extracts. This chromatographic technique requires suitable adsorbents as stationary phase that bind to target, or unwanted, compounds. Among the currently available adsorbents, silica gel has good separation ability.\textsuperscript{20–23} The major advantage of silica gel is that it presents a huge absorption capacity. Besides, silica gel is very easily available commercially in many different forms.\textsuperscript{24–26} In addition, silica gel column chromatography depends on the silica gel activity and on the polarity of the compounds.\textsuperscript{26} However, in our previous research (unpublished) we did not obtain a good separation of DHAA in a large scale using silica gel alone as the adsorbent. The reason might be that there are too many compounds in \textit{A. annua}, such as essential oils, leaf pigments, and lipids that showed polarities similar to DHAA.

Ion-exchange resin is another important adsorbent that is used in ion-exchange chromatography, an efficient separation technique for the separation of ionic or ionizable compounds.\textsuperscript{27,28} Ion exchange caused by competitive ionic interactions between similarly-charged ions and the ions fixed on the ion-exchange resins, play a critical role in the ion chromatography separation.\textsuperscript{28,29} The main benefits of ion-exchange chromatography are rapid and high-efficiency separation, low cost of consumables and large scale of production. Since its establishment, ion chromatography has been widely applied in food, agriculture, pharmaceutical, environment, biotechnology, natural products, and other fields for separation of a wide range of compounds, such as polyphenols, nucleic acid, proteins, and other organic compounds.\textsuperscript{27,30–32} However, ion-exchange chromatography has not been applied to the separation of DHAA from its complex plant matrix. DHAA contains a carboxylate group (–COOH) that could be in an ionized form when in alkaline solutions, and it can be separated as carboxylate anions by anion-exchange chromatography. Because there are many kinds of organic acids in plant cells, it is difficult to obtain high purify compounds from plant extracts under single ion exchange chromatography.

The objective of this work was to combine ion exchange resin with silica gel chromatography for the separation and purification of DHAA from APW. This combined procedure takes advantage of both chromatography methods and overcomes the disadvantages of each approach when used separately. The successful isolation of DHAA from APW can increase ART production per \textit{A. annua} cultivated area and reduce the global prices of the needed antimalarial ACT.

### Experimental

**Sample Preparation** APW was purchased from Hunan Vigor Bio-Tech Company (Loudi, Hunan Province, China) and was dissolved in 0.30\% of sodium hydroxide (NaOH) solutions at 4 mg/mL. Then, this stock DHAA solution went through serial dilutions to obtain concentrations ranging from 4 to 0.4 mg/mL.

**Anion-Exchange Resins and Pretreatment** Ten different types of anion-exchange resins (D201, D290, 201×4, 335, D301r, D264, 717, D315, D318, D314), whose properties are shown in Table 1, were purchased from the Chemical Plant of NanKai University (Tianjing, China). Before use, the resins were pretreated as follows. Firstly, the resins were soaked in 3-fold their volume of deionized water for 18–24 h. Then, the water was discarded and the resins were soaked in 1 mol/L HCl (hydrochloric acid) for 8 h, and subsequently washed with 10-fold their volume of deionized water. Finally, the resins were treated with 4\% (w/v) NaOH solution for 8 h and washed with deionized water until pH was neutral. The pretreated resins remained soaked in deionized water until needed.

**Screening of Adsorption Resins** Thirty milliliters of the pretreated anion-exchange resins were transferred into a 100 mL conical flask, and then added of 25.0 mL of a sample solution of DHAA at 4 mg/mL. Subsequently, the conical flasks were shaken at 100 rpm in a shaker at 25°C for 24 h. Before and after adsorption, DHAA concentrations in the sample were determined using GC according to the method described in section entitled “GC analysis of DHAA.” After the adsorption was completed, the anion-exchange resins were washed with 25 mL of deionized water three times. Subsequently, 25 mL of 5\% (w/v) sodium chloride (NaCl) was added and the

<table>
<thead>
<tr>
<th>Name</th>
<th>Functional groups</th>
<th>Capacity (meq/mL)</th>
<th>Pore size ((\mu)m)</th>
</tr>
</thead>
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<tr>
<td>D201</td>
<td>–N'(CH$_3$)$_3$</td>
<td>&gt;1.0</td>
<td>315–1250</td>
</tr>
<tr>
<td>D290</td>
<td>–N'(CH$_3$)$_3$</td>
<td>&gt;1.0</td>
<td>315–1250</td>
</tr>
<tr>
<td>201×4</td>
<td>–N'(CH$_3$)$_3$</td>
<td>&lt;1.2</td>
<td>315–1250</td>
</tr>
<tr>
<td>717</td>
<td>–N'(CH$_3$)$_3$</td>
<td>&lt;1.4</td>
<td>600–750</td>
</tr>
<tr>
<td>335</td>
<td>–NH$_2$</td>
<td>&gt;0.65</td>
<td>315–1250</td>
</tr>
<tr>
<td>D301r</td>
<td>–N(CH$_3$)$_2$</td>
<td>&lt;1.3</td>
<td>315–1250</td>
</tr>
<tr>
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<td>–N(CH$_3$)$_2$</td>
<td>&gt;0.65</td>
<td>600–1600</td>
</tr>
<tr>
<td>D315</td>
<td>–NHCH</td>
<td>&gt;1.0</td>
<td>315–1250</td>
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<td>–N(CH$_3$)$_2$</td>
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<td>315–1250</td>
</tr>
<tr>
<td>D314</td>
<td>–NHCH</td>
<td>&gt;1.25</td>
<td>315–1250</td>
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</table>
conical flask was shaken at 100 rpm for 24 h at 25°C. After desorption, DHAA concentration was determined by GC. The adsorption and desorption capacity, and desorption ratio of each anion-exchange resins were calculated according to Eqs. 1–3, respectively.

\[ Q_e (\text{mg/g}) = \frac{(C_0-C_e)V_0}{W} \]  

\[ Q_d (\text{mg/g}) = \frac{C_fV_d}{W} \]  

\[ D (%) = \frac{C_dV_d}{(C_0-C_d)V_0} \times 100\% \]  

where \( Q_e \) represented the adsorption capacity of each anion-exchange resin; \( Q_d \) represented the desorption capacity of each anion-exchange resin; \( D \) represented the desorption ratio; \( C_0 \) and \( C_e \) represented the initial and equilibrium concentrations of DHAA in the adsorption solutions (mg/mL), respectively; \( V_0 \) and \( V_d \) represented the adsorption and desorption solution volume (mL), respectively; \( W \) represented the dry weight of the resin (g); \( C_f \) (mg/mL) represented the DHAA concentration in the desorption solutions.

**Selection of Desorption Solvent** Four different desorption solutions, including 10% \( \text{NH}_4\text{Cl}+30\% \) ethanol; 10% \( \text{NaCl}+2\% \) NaOH; 5% \( \text{NaCl}+2\% \) NaOH; 30% ethanol; and 5% \( \text{NaHCO}_3 \), were tested in order to select the best desorption solvent for DHAA from the anion-exchange resin 717 in conical flasks. When adsorption equilibrium reached, 25 mL of above desorption solution was added to 717 resin adsorbed DHAA. Then, the conical flask was shaken at 100 rpm for 24 h at 25°C. The DHAA level in desorption solution was quantified by GC. Subsequently, the concentration of the best desorption solvent was determined by testing different combination of different concentration of \( \text{NH}_4\text{Cl} \) and ethanol.

**Establishment of Static Adsorption Kinetics** The adsorption kinetic on the 717 anion-exchange resin was determined by mixing the sample solution at 4.0 mg/mL of DHAA with 1 g of resin (dried weight) in a series of 100 mL conical flasks. The conical flasks were shaken at 25°C, at 100 rpm for 24 h. The DHAA concentrations were determined using GC at regular time intervals until equilibrium.

**Development of a Static Adsorption Isotherm** The tests to determine the adsorption isotherm of DHAA purification by 717 were conducted using 100 mL of the sample solution at different initial concentrations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 mg/mL) with 1 g of resin (dried weight) in a shaker (100 rpm) at 25, 35, and at 45°C for 24 h. When the adsorption equilibrium was reached, the adsorption capacity of DHAA on 717 anion-exchange resins was calculated according to formula 1, and their fitness to the Langmuir and Freundlich model was established using Sigma Plot 10.0 (San José, CA, U.S.A.).

The Langmuir model can be described by Eq. 5 below:

\[ Q_e (\text{mg/g}) = K_f C_e^{1/n} \]  

where \( Q_e \) represented the capacity of adsorption; \( K_f \) represented the Freundlich constant; \( C_e \) represented DHAA concentration (mg DHAA/mL) in the adsorption solution; and \( 1/n \) represented the empirical constant.

**Dynamic Adsorption and Desorption Tests** The separation characters of the DHAA on 717 were further evaluated using dynamic adsorption and desorption tests. One gram (dried weight) of pretreated 717 anion-exchange resins was added into a glass column in wet-packing way. Then the sample solution, containing 3.2 mg/mL DHAA in 0.3% NaOH solution, was pumped through the 717 column at a rate of 2 BV (bed volume) per hour. The DHAA concentration in the flow-through was quantified using GC. Once the DHAA was detected in the flow-through, addition of DHAA stopped, and adsorption was started until equilibrium was reached. Subsequently, the adsorbed anion-exchange column was washed with deionized water and desorbed with 10% \( \text{NH}_4\text{Cl}+80\% \) ethanol at a rate of 2 BV/h. The DHAA concentration in each eluate was quantified using GC-flame ionization detector (FID).

**Silica-Gel Column Chromatography** DHAA raw products (about 0.81 g) isolated from anionic exchange resin was dissolved in chloroform and poured into silica-gel (40–80 mesh) and mixed. Then, the chloroform was evaporated under vacuum at 38°C and subsequently the dried silica-gel adsorbed DHAA was loaded onto a column, installed by 8 mL of silica-gel (40–80 mesh) in a dry-packing way. Petroleum ether (PE) was used to wash the sample. Then, DHAA was eluted by increasing gradients of ethyl acetate (EtOAc) with PE (the ratio of EtOAc to PE ranged from 0 to 20%). The eluate was collected separately and quantified for DHAA using GC. The eluates containing DHAA were combined, evaporated under vacuum, and the dry product was re-dissolved in chloroform and quantified for DHAA using GC. Then, the dried sample was re-crystallized using EtOAc to obtain purified DHAA crystals.

**Scaled-Up Experiment for Validation of the Developed Method** In order to validate the developed method for separation of DHAA from APW, a scaled-up experiment was performed in triplicate using approximately 160 g of APW, 300 mL of resin 717 and 80 mL of silica-gel under the separation conditions developed in this study.

**GC Analysis of DHAA** For the quantification of DHAA in alkaline solution, the pH was adjusted to 7 or slightly below. Then, two volumes of chloroform were added for extraction of DHAA. The water in chloroform extraction solution was removed using anhydrous \( \text{Na}_2\text{SO}_4 \). Then, 1.0 µL of chloroform solution was injected into GC to quantify DHAA. For the quantification of DHAA in organic solvent, 1.0 µL of sample was injected into GC for analysis. For the quantification of DHAA in solid products, the sample was dissolved in chloroform and then was directly analyzed by GC-FID. A purified standard of DHAA was purchased from National Institute for Control of Pharmaceutical and Biologic Products (Beijing, China). The GC condition was reported previously. Briefly, an HP-5 capillary column (Agilent, 0.25 µm of film thickness, 30 m×0.32 mm i.d.) was used as stationary phase.
and using Nitrogen as the carrier gas. The injector and the detector temperature were set at 235 and 285°C, respectively. The initial oven temperature was set at 180°C; then, gradually increased to 220°C at a rate of 6°C/min, holding for 3 min at each temperature. From this point on, temperature was increased to 280°C at a rate of 30°C/min, holding at 280°C for 10 min.

Statistical Analysis All of data obtained in this study were analyzed using statistical software SPSS 10.1 and is presented as means ± standard deviation (S.D.). Statistical significance was established by ANOVA at the significance level of \( p \leq 0.05 \).

Results and Discussion

**Adsorption and Desorption Capacity, and Desorption Ratio of the Resins** Anion-exchange resins have shown excellent efficiency for separation of organic acids from complex matrices like plant extracts, and they have been applied in the purification of plant organic acids. DHAA has properties similar to an organic acid and could produce ions in alkaline solution. In order to determine which anion-exchange resin was suitable to separate DHAA from APW, 10 anion-exchange resins were investigated for their adsorption/desorption capacities. The results (Fig. 2) indicated that the anion-exchange resins 717, 201×4 and D290 presented the highest DHAA absorption capacities compared to the other resins, with adsorption capacities of 82.76, 72.07 and 71.16 mg/g, respectively. All resins had poor desorption ratios when NaCl solutions were used as the eluent solvent. Comparatively, 717 resin had the highest desorption ratio (12.14%), while 201×4 and D290 had lower desorption ratios of 4.91 and 5.69%, respectively. Hence, the 717 resin was selected as the best suitable resin for the next study.

**The Effect of Eluent Type on Desorption Capacity** Because above eluent solvent (NaCl solution) lead to a poor desorption of DHAA in the resin screening assays, different eluents were investigated in order to identify the best eluent for desorption of DHAA from anionic exchange resins. These eluents included 10% NH₄Cl + 30% ethanol, 10% NaCl + 2% NaOH, 5% NaCl + 2% NaOH, 30% ethanol and 5% NaHCO₃. Our results (Fig. 3) indicated that mixed eluents are better than a single eluent, and that the combination of 15% NH₄Cl with 30% ethanol in water had the best desorption capacity. Therefore, this eluent combination was selected for further assays.

**The Effect of NH₄Cl Concentration on Desorption Capacity** In order to further improve the desorption efficiency of DHAA from the resin, the effect of different concentrations of NH₄Cl on desorption capacity was investigated. Three concentrations of NH₄Cl (5, 10, 15%), with or without 30% ethanol, were tested. Results (Fig. 4) showed that the concentration of NH₄Cl significantly affected the desorption capacity of the
resin, and the addition of ethanol improved desorption over NH₄Cl alone. Moreover, there was no significant difference on the desorption capacity between 15% NH₄Cl+30% ethanol and 10% NH₄Cl+30% ethanol. Therefore, considering the cost:benefit ratio, 10% NH₄Cl combined with 30% ethanol was selected as the best eluent for the following assays.

**The Effect of Ethanol Concentration on Desorption Capacity** Ten percent NH₄Cl combined with different concentrations of ethanol was used as eluent. Results (Fig. 5) showed that, at first, desorption capacity increased with increasing concentration of ethanol. However, when ethanol concentration was more than 80%, the desorption capacity decreased. Therefore, 10% NH₄Cl+80% ethanol was selected as the best eluent.

**Static Adsorption and Desorption Kinetics** To optimize the separation condition of DHAA on 717 anion-exchange resin, static adsorption and desorption kinetics were analyzed at 25°C. The adsorption kinetics curves for DHAA on 717 anion-exchange resin are shown in Fig. 6A. The DHAA concentration in the solution decreased rapidly in the first 3h and subsequently stabilized, which indicated that the adsorption capacity of 717 anion-exchange resin to DHAA increased sharply with incubation time and then slowly reached adsorption equilibrium at approximately 4h (Fig. 6A). Hence, 4h is sufficient to reach the adsorption equilibrium for DHAA. By contrast, DHAA concentration in the elution solution increased rapidly at the beginning of the desorption process, but subsequently stabilized after approximately 1h, which implied that the desorption capacity of DHAA on 717 resin increased sharply at the beginning of desorption process, but slowly stabilized and was completed within 30 min (Fig. 6B).

**Adsorption Isotherms on 717 Anion-Exchange Resin** Static adsorption isotherms were investigated by adding 100 mL of a solution containing DHAA at different initial concentrations (ranged from 0.4–4.0 mg/mL) to 1 g 717 anion-exchange resin (dried weight) at 25, 35, and 45°C, respectively. The adsorption capacities of 717 sharply increased as the initial concentrations of DHAA increased from 0.4 to 1.6 mg/mL (as shown in Figs. 7A, B). After that point, the adsorption capacity increased slowly as the initial DHAA concentration increased.

![Fig. 5. The Effect of Increasing Ethanol Concentration on the Elution Efficiency of 10% NH₄Cl](image)

Error bars denote S.D. for n=3.

![Fig. 6. The Static Adsorption (A) and Desorption Kinetics (B) of DHAA on 717 Resin at 25°C](image)

![Fig. 7. Langmuir (A) and Freundlich (B) Adsorption Isotherms for DHAA onto 717 Resin at Different Temperatures](image)

One hundred milliliters of the DHAA solution at different initial concentration (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 mg/mL) with 1 g of resin (dry weight) in a shaker (100 rpm) at 25, 35, and 45°C for 24h.
continued to increase. Finally, the increase of the adsorption capacity became stable when the initial concentration of DHAA was above 3.2 mg/mL. Therefore, the concentration of DHAA of 3.2 mg/mL was used in the following assays. In addition, the temperature significantly affected the adsorption (Figs. 7A, B). The results (Figs. 7A, B) showed that the adsorption capacity decreased with increasing temperature, which indicated that the adsorption of anion-exchange resin to DHAA was an exothermic chemical process.

Based on above data (Figs. 7A, B), the analysis of correlation between adsorption capacity and initial concentration of sample at a certain temperature was performed and the equations were obtained as shown in Table 2. The high values of $R^2$ for both Langmuir and Freundlich equations to anion-exchange resin absorption for DHAA indicated that both models were suitable to describe the absorption properties of anion-exchange resin within the tested concentration range. Relatively, the adsorption properties of the anion-exchange resin for DHAA were better fitted to the Langmuir model than to the Freundlich model for each tested temperature.

**Dynamic Adsorption and Desorption** In order to optimize the dynamic adsorption and desorption processes, the dynamic adsorption and desorption processes of 717 were conducted based on the results of static adsorption and desorption experiments, which could be used to predict the adsorption and desorption characters of the resins in dynamic systems. The breakthrough curves (Fig. 8) of the 717 anion-exchange resin were established, which showed that no DHAA was detected in the eluent before 4 BV of sample was added, which indicated that the DHAA in the sample solutions was almost completely adsorbed by the 717 anion-exchange resin when the loading solution was less than 4 BV of the column. A small concentration (0.69 mg/mL) of DHAA was detected when the loading volume was 6 BV. Then, the concentrations of DHAA in the effluent liquid dramatically increased until it became static when 8 BV of sample solution were added. Thus, a sample loading of 4–6 BV was selected for the dynamic adsorption.

After finishing the adsorption, the fully saturated 717 resin was washed with 5 BV of deionized water and subsequently flushed with 10% $\text{NH}_4\text{Cl}+80\%$ ethanol in water (v/v) and the DHAA concentrations in each eluent were analyzed using GC-FID. The dynamic desorption curve of DHAA from the 717 resin at a flow rate of 2 BV/h was obtained (as shown in Fig. 9). The results of the dynamic adsorption (Fig. 9) showed that desorption of DHAA was complete once 3 BV of eluate flowed through the column. A small concentration (0.69 mg/mL) of DHAA was detected when the loading volume was 6 BV. Then, the concentrations of DHAA in the effluent liquid dramatically increased until it became static when 8 BV of sample solution were added. Thus, a sample loading of 4–6 BV was selected for the dynamic adsorption.

**Silica-Gel Column Chromatography and Crystallization**
In order to further improve the purity of DHAA, silica-gel column chromatography was conducted and followed by a gradient elution with EtOAc:PE system. Our results (Fig. 10) indicated that no DHAA was detected in the first 4BV eluent containing 100% of PE. Starting with an eluent of 1BV of 10% EtOAc in PE, the amount of DHAA in eluent increased sharply and reached the peak at 1.5BV, decreasing after that point.

**Scaled-Up Purification** In order to verify the purification method developed in the present work, the process parameters were correspondingly scaled-up to 10-fold based dynamic experiments, including 10-fold amount of material and 717 resin, silica-gel. And the purity of the products from the scaled-up separation was analyzed using GC-FID. The results (Fig. 11) proved that separation of DHAA with the 717 resin coupled with silica gel chromatography was efficient in removing all the impurity peaks that appeared in the chromatogram of the samples before separation, resulting in DHAA with a purity of up to 98%, with a recovery ratio of 80.35%. This high purity of DHAA obtained from the APW proves that the proposed separation method was efficient. Furthermore, the DHAA product obtained from anion-exchange and silica gel chromatography was recrystallized using EtOAc, resulting in about 4.128 g of DHAA crystals from 159.38 g APW, which indicated 2.59 g of DHAA crystals could be obtained from 100 g of APW using the method developed in this work.

**Conclusion**

An effective purification method of DHAA from APW was established for the first time using anion-exchange resin coupled with silica-gel column chromatography. Ten types of anion-exchange resin were tested, and the 717 anion-exchange resin had the best adsorption and desorption capacities for DHAA. Furthermore, the different desorption solvents showed highly significant differences for their desorption capacities for DHAA in 717 anion-exchange resin, and the best desorption solvent was 10% NH₄Cl+80% ethanol. The adsorption equilibrium experimental data of DHAA on 717 anion-exchange resin at different temperatures (25, 35, 45°C) were well fitted to the Langmuir and Freundlich isotherms. Finally, a separation method to obtain high-purify DHAA of 98% from ART industrial waste using anion-exchange resin...
coupled with silica-gel column chromatography is presented for the first time with a DHAA yield of 2.59%. Since DHAA can spontaneously convert into ART in vitro, the present work is of great value to increase the production of ART per cultivated area through the recycling of DHAA from *A. annua* by-product, thus providing a novel way to significantly reduce the cost of ART production.

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**Conflict of Interest** The authors declare no conflict of interest. The mention of proprietary brands and names is solely for the convenience of the reader and does not imply endorsement of the authors over similar products.

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