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# A Platelet/CMC coupled with offline UPLC-QTOF-MS/MS for screening antiplatelet activity components from aqueous extract of Danshen

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ABSTRACT

Platelets play crucial roles in thromboembolic and cardiovascular disease. The main platelets membrane receptors include adenosine diphosphate receptors, thrombin receptors, thromboxane prostanoid receptors and collagen receptors. In this study, a Platelet/CMC coupled with offline UPLC-QTOF-MS/MS system was built to screen antiplatelet activity components from aqueous extract of Danshen, which serve as an agent of antiplatelet aggregation in Traditional Chinese Medicine. Rosmarinic acid, lithospermic acid, salvianolic acid B, two isomers of salvianolic acid B, salvianolic acid C, salvianolic acid D and salvianolic acid H/I were identified as the potential antiplatelet activity components. Moreover, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid C and danshensu were tested in platelet aggregation *in vitro* assay. The results suggested their retention time was closely related to the antiplatelet aggregation activities. This study provides a rapid, effective and novel method for screening the potential antiplatelet activity components from Chinese herb medicines.



## 1. Introduction

Platelets play crucial roles in thromboembolic diseases and cardiovascular disease [1,2], such as myocardial infarction, ischemic stroke and acute coronary syndromes [2,3]. The antiplatelet therapy remains be essential in prevention and treatment. Currently, the therapeutic targets of antiplatelet major include receptors located on platelet membrane (adenosine diphosphate (ADP) receptors (e.g., P2Y purinoceptor 12 ($P2Y_{12}$)), thrombin receptors (e.g., proteinase-activated receptor 1 (PAR1)), thromboxane prostanoid (TP) receptors (e.g., TPα), collagen receptors (e.g., α2β1)) and signaling molecules intracellular (e.g., cyclooxygenase 1 (COX 1), phosphodiesterases) [2]. The antiplatelet drugs developing mainly focus on the above targets. Clopidogrel ($P2Y_{12}$ antagonist) and Aspirin (COX 1 inhibitor) are two quintessential antiplatelet drugs in clinical [2]. Several new drugs in clinical trial are designed as $P2Y_{12}$ antagonist (Ticagrelor) and PAR1 antagonist (Atopaxar) [4,5]. However, a slice of adverse effects of current antiplatelet drugs make it is still necessary to seek new therapeutic agents. Long-term use of Aspirin or clopidogrel could cause drug resistance [6,7].

A multitude of Traditional Chinese Medicines (TCMs) are considered to possess the therapeutic activity of activate blood and remove stasis. It is regarded as a treasure house for new drug discovery of antiplatelet drugs from herbal medicine. It is an effective way. Danshen, the root of *Salvia miltiorrhiza* Bge, is an indispensable herb in the TCM treatment against thromboembolic diseases and cardiovascular disease [8]. The antiplatelet aggregation function of Danshen is demonstrated in a host of pharmacological and clinical studies [9] of activating blood and removing stasis. The chemical constituents of Danshen are divided into two groups: lipophilic components (tanshinone type) and hydrophilic components (phenolic acids) [10]. Recent studies have revealed that quite a few phenolic acids components in Danshen were manifested to have antiplatelet aggregation activity. Salvianolic acid B could interact with collagen receptor α2β1 and inhibit ADP-induced platelet aggregation [11,12]. Salvianolic acid A inhibit platelet activation and arterial thrombosis through the pathway of phosphoinositide 3-kinase [13]. It is paramount to develop an effective strategy to separate and identify single phenolic acids components in herbal medicine.

Cell membrane chromatography (CMC) was first proposed by Professor L.C. He and X.D. Geng in 1996 [14]. CMC has become a rapid and effective method to screen active components from complex systems [15–18]. In this study, a Platelet/CMC coupled with offline an UPLC-QTOF-MS/MS system was built as an rapid, effec-

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tive and novel bio-analytical system for separating and identifying the potential antiplatelet activity components acting on the platelet membrane receptors from aqueous extract of Danshen and others TCMs.

## 2. Material and methods

### 2.1. Reagents and chemicals

Ticagrelor was purchased from Selleck Chemicals LLC (Houston, TX, USA). Aspirin, Adenosine 5′-diphosphate (ADP) and 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Thrombin was purchased from Beijing Solarbio Technologies Ltd. (Beijing, China). Danshensu (DSS), rosmarinic acid (RA), lithospermic acid (LA), salvianolic acid B (SAB) and salvianolic acid C (SAC) were purchased from Tianjin Zhong Xin Pharmaceutical Group Co., Ltd. (Tianjin, China). The purity of each reference chemical was above 98%. Silica gel (YQG, 5 μm, 118 Å) was obtained from Qingdao Meigao Chemical (Qingdao, China). Acetonitrile (LC-MS grade) was purchased from Honeywell Burdick & Jackson (Morristown, NJ, USA). Formic acid (HPLC grade) was purchased from Mreda Technology (Columbia, TN, USA). Other reagents used were of analytical grade. Danshen was bought from Boguang Chinese Herbal Medicine Co., Ltd. (Bozhou, China). The ultrapure water used for preparation of all aqueous solutions was produced by Milli-Q water purification system made by Millipore (Bedford, MA, USA). Polytetrafluoroethylene (PTFE) membranes of 0.22 μm used for processing samples were also purchased from Millipore.

### 2.2. Instrument configuration and conditions

Agilent 1260 Infinity Quaternary HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) was adopted in the CMC. The HPLC system comprised of a quaternary pump (G1311C), an autosampler (G1329B), a thermostat (G1330B), a thermostatted column compartment (G1316A) and a diode-array detector (G1315D). Data collection and processing were performed on Agilent 1200 Chemistation. ODS columns (4.6 mm × 12.5 mm I.D., 5 μm, Welch Materials, shanghai, China) were used to collect the retained fractions.

Waters ACQUITY™ Ultraperformance LC (UPLC) system (Waters Corporation, Milford, MA, USA) was used to separate and identify the components in retained fractions. The UPLC system was equipped with a PDA detector, a column compartment, a sampler manager and a binary solvent manager, and connected to a Xevo™ G2-S QTOF mass spectrometer. The separation was carried out on an ACQUITY UPLC™ BEH $C_{18}$ column (2.1 mm × 50 mm, I.D., 1.7 μm, Waters Corporation) at 30 °C with a flow rate of 0.3 mL/min. Mobile phase was a mixture of 0.1% formic acid–water (A) and acetonitrile (B). The gradient program of mobile phase for Ticagrelor was as follows: 0-10 min, 5-95% B. The gradient program of mobile phase for aqueous extract of Danshen was as follows: 0–3 min, 2% B; 3–5 min, 2–5% B; 5–9 min, 5–7% B; 9–10 min, 7–10% B; 10–20 min, 10–12% B; 20–21 min, 12–13% B; 21–26 min, 13–15% B; 26–30 min, 15–18% B; 30–36 min, 18–20% B; 36–38 min, 20% B; 38–41 min, 20–95% B. The injection volume was 5 μL and the PDA detection was performed in the range of 210–400 nm. The MS was operated in negative mode, with capillary to 2500 V, the cone set to 40 V, and source offset to 80 V. The desolvation gas was maintained at 600 L/h at a temperature of 400 °C. The cone gas was 50 L/h with a source temperature of 100 °C. The scan range was 100–1100 Da.

Platelet aggregation assay used FlexStation 3 (Molecular Devices Corp., Sunnyvale, CA, USA).

### 2.3. Preparation of samples and standard solutions

The aqueous extract of Danshen was prepared as follows: 50 g of Danshen was refluxed with 400 mL water for 2 h and filtered. The filtrate was concentrated to a volume of 70 mL under reduced pressure and vacuum, and then injected into preparative HPLC to remove most SAB, which was located by comparing the retention time with the standard compound. The prepared aqueous extract was concentrated and then dried by vacuum freeze-drying. 10 mg aqueous extract of Danshen (AED) dissolved in 10 mL 50% methanol–water (v/v) and then stored at −80 °C in the dark before use.

Ticagrelor, Aspirin, DSS, RA, LA, SAB and SAC standard solutions (1 mg/mL) were freshly prepared in 50% methanol–water (v/v) every week and stored at −80 °C in the dark.

For CMC analysis, all the stored solutions were diluted to suitable concentrations by mobile phase. For UPLC-QTOF-MS/MS analysis, Ticagrelor and Aspirin stored solutions were diluted with 50% methanol–water (v/v), respectively. DSS, RA, LA, SAB and SAC stored solutions were diluted with 25% methanol–water (v/v), respectively. The enriched Ticagrelor by ODS column in CMC was dissolved in 50% methanol–water (v/v). The enriched AED by ODS column in CMC was dissolved in 25% methanol–water (v/v).

All the injecting samples were filtered through a 0.22 μm PTFE membrane before analysis.

### 2.4. Platelet extract and preparation for the Platelet/CMC system

Ten male Sprague-Dawley (SD) rats (250–280 g bodyweight) were purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China). In asepsis condition, rats were anesthetized by 10% chloral hydrate and draw blood from abdominal aorta. The whole blood and ACD (38 mM Citric acid, 75 mM sodium citrate, 124 mM dextrose) 20:3 (V/V) mixture was centrifuged at 200 × *g* at room temperature for 10 min. The supernatant platelet-rich plasma was collected and centrifuged at 800 × *g* at room temperature for 10 min. The pellet was re-suspended in buffer A (pH 7.40, 130 mM NaCl, 10 mM sodium citrate, 9 mM $NaHCO_3$, 6 mM dextrose, 0.9 mM $MgCl_2$, 0.81 mM $KH_2PO_4$, 10 mM Tris-base) and then centrifuged again at 800 × *g*, 4 °C for 10 min to obtain pure platelet. The pure platelet was re-suspended in 50 mM Tris–HCl (pH 7.40) to be ruptured by ultrasonicating for 30 min, and centrifuged at 1000 × *g*, 4 °C for 10 min. The supernatant was centrifuged twice at 12,000 × *g*, 4 °C for 20 min to get platelet membrane pellet. The platelet membrane pellet was suspended in 5 mL ultrapure water. A total of 50 mg silica gel was activated at 105 °C for 30 min. The activated silica gel was slowly added into the platelet membrane suspension under vacuum at 4 °C. The mixture was subsequently agitated for 30 min with a magnetic stirrer and let stand for 12 h. The silica gel surrounding by platelet membrane was packed into the CMC column (10 mm × 2.0 mm I.D.) by a wet packing method to obtain a platelet cell membrane stationary phase column.

### 2.5. System suitability of the Platelet/CMC offline UPLC-QTOF-MS/MS system

In order to verify the system suitability of the Platelet/CMC column, the specificity, selectivity and reproducibility were tested. After optimizing the chromatographic conditions, ultrapure water was chose as the mobile phase with the flow rate of 0.1 mL/min, the detection wavelength was 254 nm, 280 nm and 293 nm, the column temperature was 37 °C ± 0.5 °C. Then the column was attached to HPLC system, equilibrated 80 min.

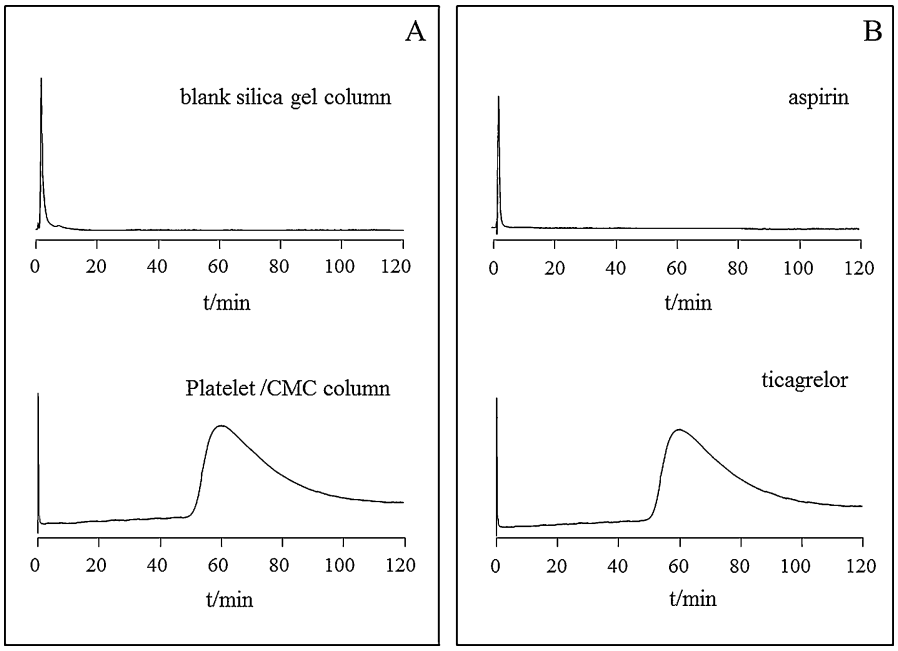


**Fig. 1.** The specificity and selectivity of the Platelet/CMC column. (A) Ticagrelor retained on different columns; (B) different antiplatelet drugs acting on the Platelet/ CMC column.

The specificity of the Platelet/CMC column was investigated by injecting ADP ($P2Y_{12}$) antagonist Ticagrelor into a blank silica gel column and a Platelet/CMC column. The different chromatograms were recorded and compared. Their chromatographic retention was used to investigate the specificity of the Platelet/CMC column.

The selectivity of the Platelet/CMC column was investigated by injecting different type antiplatelet drugs. The COX 1 inhibitor Aspirin and Ticagrelor were injected into the same Platelet/CMC column, respectively. The different chromatograms were recorded and compared. Their chromatographic retention was used to investigate the selectivity of the Platelet/CMC column.

The reproducibility of the single Platelet/CMC column and different Platelet/CMC columns was investigated, and the retention time of Ticagrelor was used as an indicator. Reproducibility of single platelet/CMC column was determined by repeated six times injection of Ticagrelor (5 μL, 0.1 mg/mL). For different platelet/CMC columns, Ticagrelor (5 μL, 0.1 mg/mL) was injected into 3 different platelet/CMC columns, respectively. The column lifetime was also tested by repeatedly injecting 5 μL Ticagrelor into a same platelet/CMC column everyday during four days.

The standard solution of Ticagrelor was adopted to verify the ability of the Platelet/CMC offline UPLC-QTOF-MS/MS system to separate and identify antiplatelet active components acting on the platelet membrane receptors. Ticagrelor (5 μL, 0.1 mg/mL) was injected into the Platelet/CMC column. The retained fractions were enriched with ODS column and then eluted with methanol. The methanol elution was evaporated to dryness under nitrogen. The enriched fractions were preparation as Section 2.3 and then injected into the UPLC-QTOF-MS/MS system for separation and identification. The UPLC-QTOF-MS/MS conditions were taken as Section 2.2.

### 2.6. Screening antiplatelet active components from AED

The Platelet/CMC offline UPLC-QTOF-MS/MS system was adopted to screen antiplatelet active components acting on the platelet membrane receptors from AED. Chromatographic conditions were taken as Section 2.5 and UPLC-QTOF-MS/MS conditions were taken as Section 2.2. AED was injected into the Platelet/CMC column. The retained fractions were enriched with ODS column and then eluted with methanol. The methanol elution was evaporated to dryness under nitrogen. The enriched fractions were preparation as Section 2.3 and then injected into the UPLC-QTOF-MS/MS system for separation and identification.

DSS, RA, LA, SAB and SAC solutions were injected into the Platelet/CMC system as external standard and their retention time were recorded.

### 2.7. Platelet aggregation assay

The platelet was prepared as Section 2.4 and was re-suspended in buffer A and adjusted to a consistent concentration (OD value 1.0). All tested compounds were dissolved in DMSO and diluted with buffer A to 50 μM. Buffer A was used as negative control. According to the types of membrane receptors, different agonists were used to induce platelet aggregation. The agonists include thrombin (activator of thrombin receptors), ADP (activator of ADP receptors) and U46619 (activator of TP receptors). The platelet aggregation assay was performed at 37 °C.

100 μL platelet suspension at a concentration of $10^8$ platelet/mL was adopted in the platelet aggregation assay on a transparent 96-well plate. 50 μL samples were added into each wells, meanwhile same amount of buffer A was added in negative control group. 50 μL agonist (0.5 U/mL thrombin [19], 25 μM ADP [13] or 4 μM U46619 [13]) were added into each wells after pre-incubation at 37 °C for 10 min under sustained oscillation. The OD values at 405 nm were recorded every 45 s during 30 min by FlexStation 3 plate reader under sustained oscillation.

$A_t = \frac{OD_0 - OD_t}{OD_t} \times 100\%(1)$

Inhibition rate $= \frac{A_{Nmax} - A_{Tmax}}{A_{Nmax}} \times 100\%(2)$$OD_0$ is the OD value at start and $OD_t$ is the OD value recorded every 45 s by plate reader. Platelet aggregation rate ($A_t$) was calculated by Eq. (1). $A_{Nmax}$ is the maximum value in all the $A_t$ of negative control and $A_{Tmax}$ is the maximum value in all the $A_t$ when the tested compounds were added. The inhibition rate of the tested compounds was calculated by Eq. (2).

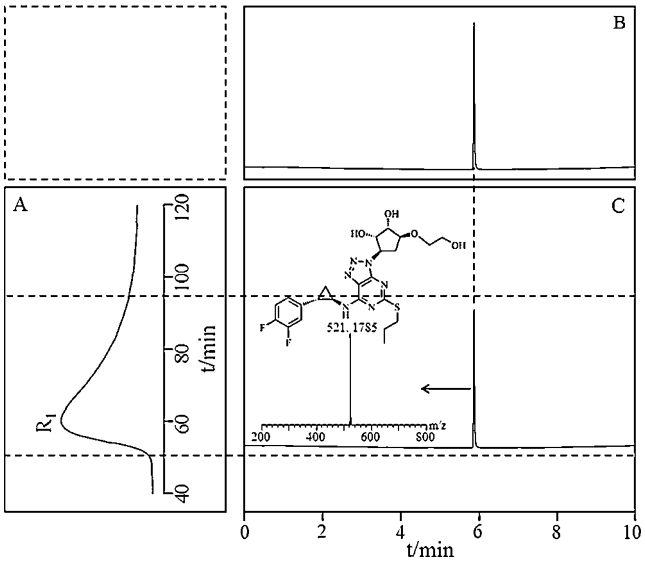


**Fig. 2.** Chromatograms of Ticagrelor standard solution analyzed by the Platelet/CMC offline UPLC-QTOF-MS/MS system. (A) The Platelet/CMC chromatogram of Ticagrelor standard solution; (B) the direct UPLC-QTOF-MS chromatograms of Ticagrelor standard solution in negative ion mode; (C) the UPLC-QTOF-MS chromatograms of retained fraction $R_1$ in negative ion mode.

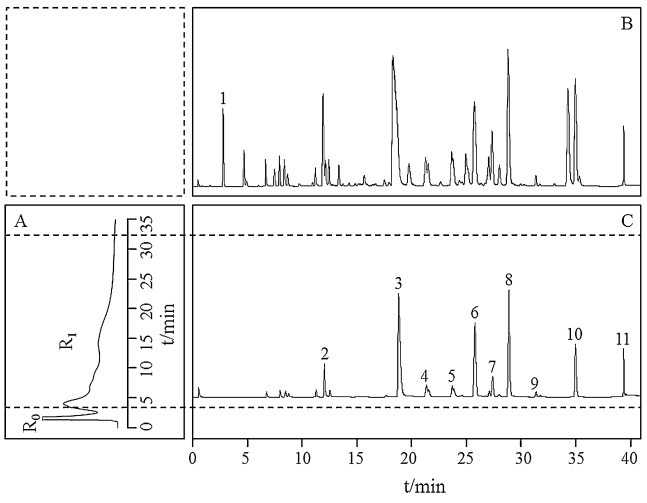


**Fig. 3.** Chromatograms of AED analyzed by the Platelet/CMC offline UPLC-QTOF-MS/MS system. (A) The Platelet/CMC chromatogram of AED including non-retained fraction $R_0$ and retained fraction $R_1$ (between the two dotted lines); (B) the direct UPLC-QTOF-MS chromatograms of AED in negative ion mode; (C) the UPLC-QTOF-MS chromatograms of retained fraction $R_1$ in negative ion mode.

## 3. Results and discussion

### 3.1. System suitability of the Platelet/CMC offline UPLC-QTOF-MS/MS system

Ticagrelor acting on the $P2Y_{12}$ receptor could be well retained on the Platelet/CMC column, but could not be retained on the blank

**Table 1**
UPLC-QTOF-MS/MS information of compounds in AED.

| Peak no. | $t_R$ (min) | Name | Formula | selected ion | Expected | Detected | Error (ppm) | MS/MS |
|---|---|---|---|---|---|---|---|---|
| 1[a,b] | 2.68 | DSS [20] | $C_9H_{10}O_5$ | $[M-H]^-$ | 197.0450 | 197.0450 | 0 | 179.0343$[M-H-H_2O]^-$, 135.0443$[M-H-H_2O-CO_2]^-$ |
| 2[c] | 11.91 | Unknown | – | $[M-H]^-$ | – | 799.1174 | – | 619.0752, 519.0926,493.1131, 439.0334, 339.0508, 321.0404, 295.0613, 269.0820, 197.0458 |
| 3[c] | 18.53 | Unknown | – | $[M-H]^-$ | – | 799.1174 | – | 619.0751, 519.0924, 493.1130, 439.0330, 339.0508, 321.0404, 295.0613, 269.0820, 197.0458 |
| 4[b,c] | 21.09 | SAH/I [20] | $C_{27}H_{22}O_{12}$ | $[M-H]^-$ | 537.1033 | 537.1027 | −1.1 | 339.0509$[M-H-C_9H_{10}O_5]^-$, 295.0611$[M-H-C_9H_{10}O_5-CO_2]^-$ |
| 5[b,c] | 23.46 | SAD [20] | $C_{20}H_{18}O_{10}$ | $[M-H]^-$ | 417.0822 | 417.0821 | −0.2 | 197.0459$[M-H-C_{11}H_8O_5]^-$, 175.0404$[M-H-CO_2-C_9H_{10}O_5]^-$ |
| 6[a,b,c] | 25.57 | RA [20] | $C_{18}H_{16}O_8$ | $[M-H]^-$ | 359.0767 | 359.0774 | 1.9 | 197.0461 $[M-H-C_9H_6O_3]^-$, 179.0354 $[M-H-C_9H_6O_3-H_2O]^-$, 161.0249$[M-H-C_9H_{10}O_5]^-$, 135.0455$[M-H-C_9H_6O_3-H_2O-CO_2]^-$, 133.0299$[M-H-C_9H_{10}O_5-CO]^-$ |
| 7[b,c] | 27.18 | ISAB1 | $C_{36}H_{30}O_{16}$ | $[M-H]^-$ | 717.1456 | 717.1454 | −0.3 | 519.0930$[M-H-C_9H_{10}O_5]^-$, 339.0511$[M-H-C_9H_{10}O_5-C_9H_8O_4]^-$, 321.0406$[M-H-2C_9H_{10}O_5]^-$, 295.0613$[M-H-C_9H_{10}O_5-C_9H_8O_4-CO_2]^-$ |
| 8[a,b,c] | 28.69 | LA [20] | $C_{27}H_{22}O_{12}$ | $[M-H]^-$ | 537.1033 | 537.1036 | 0.6 | 493.1138$[M-H-CO_2]^-$, 313.0720$[M-H-CO_2-C_9H_8O_4]^-$, 295.0618$[M-H-CO_2-C_9H_{10}O_5]^-$ |
| 9[a,b,c] | 31.24 | SAB [20] | $C_{36}H_{30}O_{16}$ | $[M-H]^-$ | 717.1456 | 717.1444 | −1.7 | 519.0925$[M-H-C_9H_{10}O_5]^-$, 339.0510$[M-H-C_9H_{10}O_5-C_9H_8O_4]^-$, 321.0403$[M-H-2C_9H_{10}O_5]^-$, 295.0610$[M-H-C_9H_{10}O_5-C_9H_8O_4-CO_2]^-$ |
| 10[b,c] | 34.79 | ISAB2 | $C_{36}H_{30}O_{16}$ | $[M-H]^-$ | 717.1456 | 717.1450 | −0.8 | 519.0927$[M-H-C_9H_{10}O_5]^-$, 339.0509$[M-H-C_9H_{10}O_5-C_9H_8O_4]^-$, 321.0406$[M-H-2C_9H_{10}O_5]^-$, 295.0611$[M-H-C_9H_{10}O_5-C_9H_8O_4-CO_2]^-$ |
| 11[a,b,c] | 39.41 | SAC [20,21] | $C_{26}H_{20}O_{10}$ | $[M-H]^-$ | 491.0978 | 491.0977 | −0.2 | 311.0557$[M-H-C_9H_8O_4]^-$, 293.0454$[M-H-C_9H_{10}O_5]^-$, 267.0656$[M-H-C_9H_8O_4-CO_2]^-$ |

[a] Compared with reference standards.
[b] Previously reported from *Salvia* species.
[c] The retained fraction.

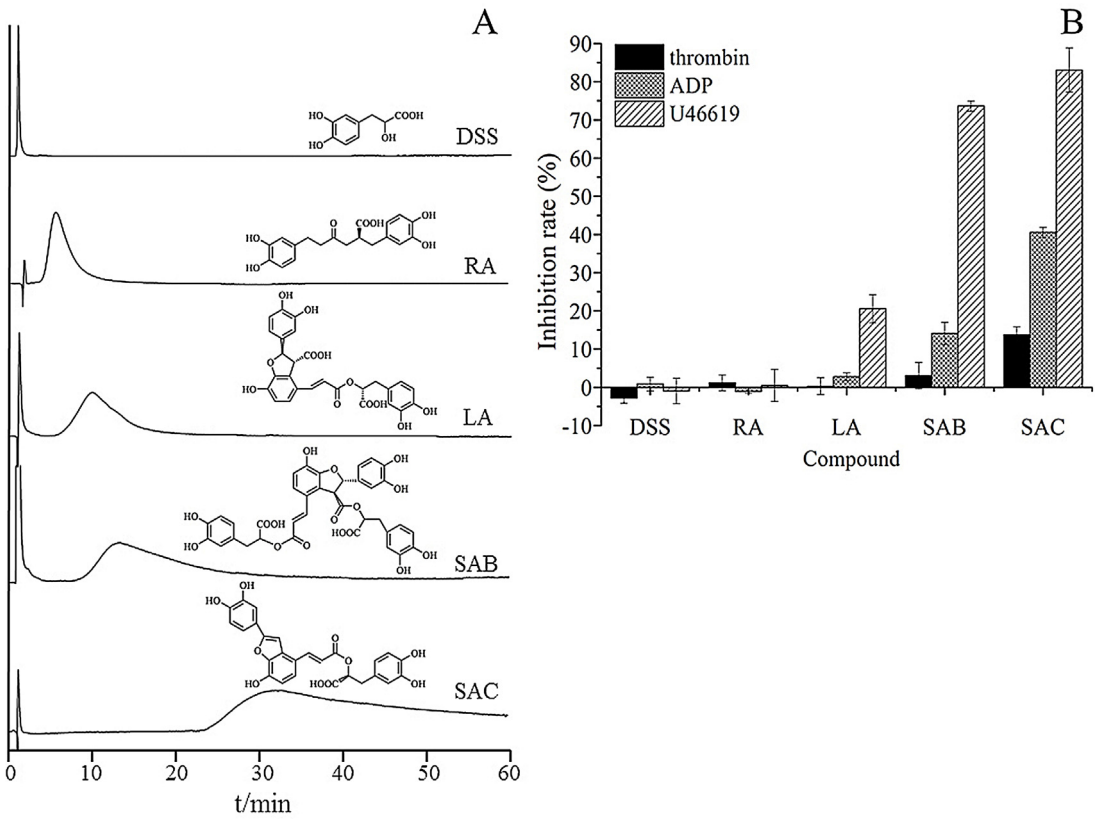


**Fig. 4.** Chromatograms and platelet aggregation inhibition rate of identified compounds in AED. (A) The Platelet/CMC chromatograms of identified compounds in AED; (B) the platelet aggregation inhibition rate of the identified compounds in AED.

silica gel column (Fig. 1A). The results suggested the Platelet/CMC column has good specificity. In Fig. 1B, Ticagrelor could be retained on the Platelet/CMC column. However Aspirin acting through the COX 1 pathway could not be retained on the Platelet/CMC column. The results suggested the Platelet/CMC column was suitable for recognizing components acting on the platelet membrane receptors.

The results of reproducibility showed that the relative standard deviation (RSD) of the retention time of different Platelet/CMC columns was 2.9% ($n = 3$), meanwhile the retention time of single Platelet/CMC column was 1.3% ($n = 6$). The experimental results of the Platelet/CMC column were reproducible. The column lifetime was a paramount parameter since the Platelet/CMC column has biological activity. Ticagrelor was retained on the Platelet/CMC column well after the CMC column was used for four days.

As shown in Fig. 2A, the retained fraction $R_1$ (between the two dotted lines) was recognized on the Platelet/CMC column. The UPLC-QTOF-MS chromatograms of ticarelor and $R_1$ in negative ion mode are shown in Fig. 2B and C, respectively. By comparing the retention time and mass information, $R_1$ was identified as Ticagrelor. The established Platelet/CMC coupled with offline UPLC-QTOF-MS/MS system gave the indispensable qualitative information accurately. Accordingly, the Platelet/CMC offline UPLC-QTOF-MS/MS system was suitable for separating and identifying antiplatelet active components acting on the platelet membrane receptors.

### 3.2. Screening antiplatelet active components from AED

SAB was proved to have antiplatelet aggregation activity and interact with membrane receptor [11,12]. To avoid the CMC, column was saturated by the high concentration of SAB, most SAB was removed by preparative HPLC from the whole aqueous extract of Danshen before load on the Platelet/CMC column (see Section 2.3). As shown in Fig. 3A, the non-retained fraction $R_0$ and the significant retained fraction $R_1$ (between the two dotted lines) were recognized on the Platelet/CMC column. The UPLC-QTOF-MS chromatograms of AED and $R_1$ in negative ion mode were shown in Fig. 3B and C, respectively. The offline UPLC-QTOF-MS qualitatively determined 10 compounds in fraction $R_1$ (Fig. 3C) successfully. Their mass information was shown in Table 1. Eight of them were identified as RA, LA, SAB, two isomers of SAB (ISAB1 and ISAB2), SAC, salvianolic acid D (SAD) and salvianolic acid H/I (SAH/I). Others chromatographic techniques were inevitable in the structures elucidation of the other two compounds. As shown in Fig. 3B, a lot of compounds in AED including DSS were not retained on Platelet/CMC column.

In order to verity the chromatographic retention of DSS, RA, LA, SAB and SAC, their standard solutions were injected into the Platelet/CMC column. The results are shown in Fig. 4A. It was revealed that DSS was not retained, RA, LA, SAB and SAC were retained. The retention time sequence was DSS < RA < LA < SAB < SAC.

### 3.3. Platelet aggregation assay

*In vitro* platelet aggregation assay was performed to test the antiplatelet aggregation activity of the retained and non-retained compounds including RA, LA, SAB, SAC and DSS. The results are shown in Fig. 4B and Table 2, the sequence of antiplatelet aggregation activities was $0 \approx$ DSS $\approx$ RA < LA < SAB < SAC. In Section 3.2, the retention time sequence was DSS < RA < LA < SAB <SAC. The results suggested their retention time was closely related to the antiplatelet aggregation activities.

**Table 2**
Platelet aggregation inhibition rate of the identified compounds in AED.

| Compound | Inhibition rate (%) | | |
|---|---|---|---|
| | thrombin-induced | ADP-induced | U46619-induced |
| DSS | −2.81 ± 1.35 | 0.89 ± 1.79 | −0.95 ± 3.33 |
| RA | 1.19 ± 2.06 | −1.08 ± 0.50 | 0.50 ± 4.21 |
| LA | 0.30 ± 2.19 | 2.79 ± 1.03 | 20.56 ± 3.71 |
| SAB | 3.05 ± 3.46 | 14.10 ± 2.88 | 73.62 ± 1.32 |
| SAC | 13.76 ± 2.09 | 40.55 ± 1.36 | 83.03 ± 5.78 |

## 4. Conclusion

In summary, a Platelet/CMC coupled with offline UPLC-QTOF-MS/MS system was built successfully to screen antiplatelet activity components from aqueous extract of Danshen. RA, LA, SAB, two isomers of SAB, SAC, SAD and SAH/I were identified as the potential antiplatelet activity components. The results of platelet aggregation assay suggested retention time of 5 compounds (DSS, RA, LA, SAB and SAC) was closely related to the antiplatelet aggregation activities. It is believed that the developed Platelet/CMC offline UPLC-QTOF-MS/MS system is novel and viable strategy for screening the potential antiplatelet activity components from TCMs and herbal medicine.

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