Simultaneous determination of three naphthoquinones from *Arnebia euchroma* (Royle) Johnst in beagle plasma by UPLC-MS/MS and application for pharmacokinetics study

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**ABSTRACT**

A rapid, simple and efficient ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) method was established to simultaneous determination of shikonin, isobutyryl shikonin, β, β’-dimethylacryl alkanin in beagle plasma and evaluated by using esculetin as internal standard. The electrospray ionization (ESI) source was operated in negative ionization mode. Multi-reaction monitoring (MRM) was used to quantitatively analyzed shikonin \( m/z \) 287.0 → 217.9, isobutyryl shikonin \( m/z \) 357.0 → 268.9, β, β’-dimethylacryl alkanin \( m/z \) 370.0 → 270.1 and esculetin \( m/z \) 177.0 → 89.0, respectively. The method was validated for selectivity, linearity, lower limit of quantification, precision, accuracy, recovery, matrix effect and stability. All validation parameters met the acceptance criteria according to regulatory guidelines. This method was successfully applied for the pharmacokinetic study of shikonin, isobutyryl shikonin, β, β’-dimethylacryl alkanin in beagle dogs plasma after oral administration of *A. euchroma* extract.

**KEYWORDS**

*Arnebia euchroma* (Royle) Johnst, pharmacokinetics, naphthoquinones

**INTRODUCTION**

*Arnebia euchroma* (Royle) Johnst (*A. euchroma*) is a kind of perennial herb of the arnbiaceae genus belong to boraginaceae family. This plant is recorded for the first time in Shennongbencaojing [1, 2] and is widely distributed in Indian, Japan and other Asian countries, especially the arid regions of Asia and North Africa and it was widely used in China [3–6]. According to the Chinese Pharmacopoeia (2015 edition), decoction pieces of *A. euchroma* can be used in clearing heat and cooling blood, promoting blood circulation and detoxication, eliminating eruption and plaques. Soaking in 5–10g boil cream or vegetable oil for external use [7]. Previous studies have reported that *A. euchroma* contains various bioactive compounds especially naphthoquinones, including β-hydroxyisovalerylshikonin [8], shikonin [9], teracrylshikonin, acetylshikonin [10], β, β’-dimethylacrylic acanin, isobutyrylshikonin, α-methylbutyrylshikonin, isovalerylshikonin, dehydroacanin, and a small amount of deoxyshikonin [11, 12]. Naphthoquinones are considered to be active ingredients in herbal medicine and possess a broad range pharmacological properties, including promoting wound healing [13], anti-hiv activity [14], anti-inflammation [15, 16], anti-tumor [17], antioxidant [18, 19], antivirus [20], antibacteria [21] and so on. Pharmacological studies
have revealed that the three components have several similar pharmacological activities, including antivirus [22], antioxidant [23], anti-inflammation [24], enhanced immunity [25] and antifertility [26]. In recent years, several analytical methods based on chromatographic techniques have been used for the determination of shikonin, isobutryl shikonin and β, β’-dimethylacryl alkalin from A. euchroma. An analytical method based on NP-HPTLC was developed to simultaneous determine of shikonin and β, β’-dimethylacryl shikonin of A. benthmaii root in vitro [27]. The LC-ESI-MS/MS method was used to determine the pharmacokinetics of shikonin [29]. In order to provide the basis for the future clinical application of these components, it makes great sense to establish a method to learn the pharmacokinetics behavior of shikonin, isobutryl shikonin and β, β’-dimethylacryl alkalin in beagle dogs plasma.

Ultra-high performance liquid chromatography (UPLC) is a commonly used chemical component separation technology, which has many advantages such as high speed and high detection sensitivity. In this paper, it was the first applied to research pharmacokinetic characteristic of three components from A. euchroma in beagle dogs plasma, especially the pharmacokinetics of isobutryl shikonin and β, β’-dimethylacryl alkalin was firstly detected.

**EXPERIMENTAL**

**Chemicals and reagents**

Methanol and acetonitrile were purchased from Fisher Corporation in Massachusetts (USA). Formic acid was obtained from Dikma Corporation in Massachusetts (USA). Sodium hydroxide and hydrochloric acid were supplied by Tianjin Continental Chemical Reagent Factory (China). Distilled water was purchased from Watsons Group Co., Ltd. In Hong Kong (China) and disposable vacuum blood-collecting vessel were provided by Jiangxi Zhongjie Medical device Co., Ltd (China). The reference standards for shikonin, isobutryl shikonin and β, β’-dimethylacryl alkalin were isolated in laboratory and Esculetin was selected as the internal standard (IS) for shikonin and isobutryl shikonin, β, β’-dimethylacryl alkalin and IS are shown in Fig. 1.

Preparation of standards and quality control (QC) samples

Weighing A. euchroma 100g and extracted by ultrasonic extraction with 5 times the amount of petroleum ether, extracted twice for 30 min each time. The extract of it was obtained by filtration, combined with filtrate, concentration by decompress and recovery of petroleum ether. A mixed stock solution was prepared with methanol and the concentration were 520 μg mL⁻¹ for shikonin, 400 μg mL⁻¹ for isobutryl shikonin and 560 μg mL⁻¹ for β, β’-dimethylacryl alkalin, respectively. The final concentrations of shikonin were 4.06, 8.11, 32.43, 64.87, 129.7, 259.5, 519.0 ng mL⁻¹, isobutryl shikonin were 3.18, 6.29, 25.18, 50.37, 100.7, 201.5, 403.0 ng mL⁻¹ and β, β’-dimethylacryl alkalin were

![Table 1. Optimized mass spectrometric parameters for analytes and IS](image)

| Analytes                    | Precursor ion (m/z) | Product ion (m/z) | Declustering potential (V) | Collision Energy (V) | Polarity |
|-----------------------------|--------------------|------------------|---------------------------|----------------------|----------|
| shikonin                    | 287.0              | 217.9            | −100.50                   | −16.71               | negative |
| isobutryl shikonin          | 357.0              | 268.9            | −108.07                   | −17.68               | negative |
| β, β’-dimethylacryl alkalin | 370.0              | 270.1            | −54.80                    | −14.86               | negative |
| esculentin                  | 177.0              | 89.0             | −69.26                    | −32.92               | negative |
4.37, 8.76, 35.06, 70.12, 140.2, 280.5, 561.0 ng mL⁻¹ were prepared by further dilution of the mixed stock solution with methanol. The QCs at four concentration levels including lower limit of quantitation (LLOQ), low quality control (LQC), middle quality control (MQC) and high quality control (HQC) were 4.06, 9.73, 64.87, 415.2 ng mL⁻¹ for shikonin, 3.18, 7.55, 50.37, 322.4 ng mL⁻¹ for isobutyryl shikonin and 4.37, 10.51, 70.12, 448.8 ng mL⁻¹ for \( \beta, \beta' \)-dimethylacyr alkanin, respectively. Esculetin was used as internal standard and diluted with methanol to form a solution with a final concentration of 250.0 ng mL⁻¹. All the stock and standard working solutions were stored at 4°C before use.

**Plasma samples preparation**

100 µL plasma sample and 50 µL 10% hydrochloric acid were added in a dry 10 mL glass tube, vortex mixed for 1 min, then added 100 µL internal standard solution and 200 µL methanol solution vortex for 1 min and added 200 µL ethyl acetate. The tube was vortex for 1 min and centrifuged at 15,000 rpm for 10 min. Supernatant was transferred to a
new glass tube and added NaOH to adjust liquid to pH 4, then evaporated to dryness under nitrogen stream at 40°C. The residue was reconstituted with 100 μL of initial mobile phase, then vortexed for 3 min, sonicated for 2 min and centrifuged at 12,000 rpm for 10 min. Finally, a 10 μL aliquot was injected into the UPLC-MS/MS system for analysis.

**Method validation**

The developed UPLC-MS/MS method was validated according to the US Food and Drug Administration Bioanalytical Method Validation Guide [28]. Validation contents include selectivity, linearity, lower limit of quantitation, precision, accuracy, recovery, matrix effect, and stability.

**Selectivity.** Taking blank plasma from 6 different beagle dogs, chromatograms of the blank plasma, plasma with reference substance (LLOQ) and internal standard and 1.5 h after reference substance (LLOQ) and internal standard, quality control sample (QC) and internal standard were compared. The purpose of the determination is to investigate whether the intrinsic interference exists in the detection of the analytes, retention time of internal standard and responsibility.

**Linearity and LLOQ.** The mixed standard solutions of shikonin, isobutyryl shikonin, β, β’-dimethylacryl alkanin and blank plasma were taking 100 μL in 10 mL glass tube respectively. The samples were processed and analyzed according to section “plasma samples preparation”. Record the peak area of the three compounds and internal standard at different concentrations and calculate the peak area ratio. The abscissa (X) is the concentration of 3 compounds, while the ordinate (Y) is the peak area ratios of compounds to internal standard. Using the weighted least square method (1/x² as weighing factor) to calculate the linear regression and draw the standard curve of compounds. LLOQ is the minimum concentration on the standard curve and the signal-to-noise ratio (S/N) is required to be about 10. The accuracy and precision of LLOQ should be within a reasonable and acceptable range, the relative error (RE) should be less than 20% and the relative standard deviation (RSD) should be in the range of ±20%.

**Precision and accuracy.** The blank plasma and QC samples with low, medium and high concentrations were taken 100 μL, respectively. The plasma samples were treated according to the section “plasma samples preparation”. Six samples were prepared of each concentration and determined for 3 days and recorded the peak area, calculated the peak area ratio of 3 compounds to internal standard and the standard curve was drawn. Calculated the concentration of QC sample within the same day. Then obtained the accuracy and precision of Intra-day and Inter-day. The RE should be less than 15%, and the RSD was required to be within ±15%.

**Recovery and matrix effect.** Taking 100 μL blank plasma and the QC samples with low, medium and high concentrations were processed as mentioned in section “plasma samples preparation”. The peak area ratio of compounds to internal standard at each concentration was measured and calculated (A). 100 μL blank plasma was treated according to the plasma samples preparation method. The residue was redissolved with QC samples respectively after evaporated to dryness under nitrogen stream. The ratio peak area of 3 compounds to internal standard (B) at each concentration was measured and calculated. QC samples were directly injected and analyzed, recording the peak area ratio of compounds to internal standard (C). The above low, medium and high concentrations of QC samples were analyzed with 6 samples of each concentration.

**Stability experiments.** Taking 100 μL blank plasma and QC samples with low, medium and high concentrations were treated according to the section “plasma samples preparation”. Each concentration was determined with 6 samples and investigated the stability under the following different storage conditions: Short-term stability (24 h at room temperature); long-term stability (2 weeks at −20°C), freeze-thaw cycle stability (3 cycles between −20°C and room temperature), post-preparation stability (24 h in the sample disk of the automatic sampler).

**Application of the method to pharmacokinetic studies**

Six adult healthy male beagle dogs (weight 12–14 kg) were purchased from Shenyang Kangping (China) Laboratory Animal Research Institute (Animal Certificate: scxk (Liao) 2014-0003). Adequate food and water were given under the condition of constant temperature and humidity (25 ± 2°C, 60 ±10%), fasting for 12 h and drinking water was free before the experiment.

The dosage of beagle dog was 267.1 mg kg⁻¹. Using HPLC to determine the content of shikonin, isobutyryl shikonin and β, β’-dimethylacryl alkanin is 1.48, 2.38, 4.19 mg g⁻¹, respectively. The extract was administered as capsules without food and blood samples were collected into heparinized tubes pre-dose and at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h post-dose. The samples was centrifuged at 3,500 rpm for 10 min, the supernatant was placed in a 1.5 mL EP tube and stored at −20°C.

Several pharmacokinetic parameters of the 3 compounds were calculated with DAS 2.0 and drawn the plasma concentration-time curve by non-atroventricular model. The pharmacokinetics parameters included maximum plasma concentration (C_max), time to reach the maximum concentrations (T_max), half-life (T_1/2) and the area under plasma concentration–time curve (AUC). C_max and T_max can be observed directly on the curve. T_1/2 can be calculated by using the formula T_1/2 = 0.693/Kc and the area under the plasma concentration-time curve to time infinity (AUC_{0→∞}) was calculated as AUC_{0→∞} = AUC_{0→t} + C/C/Kc. All the calculated results were expressed as Mean ±SD.
RESULTS AND DISCUSSION

Optimization of the UPLC-MS/MS conditions

The type of mobile phase plays a key role in the chromatographic analysis of the compounds and affect the separation degree and ionization effect of the compounds. In this article, we investigated the acetonitrile-water system and the methanol-water system. The results show that when the organic phase is acetonitrile, the separation degree and the retention time is stable and the noise interference is low. Therefore, acetonitrile was selected as the organic phase in the mobile phase. Meanwhile, the effect of different proportion of formic acid on the peak shape and response value of the mobile phase was also investigated. The results showed that when 0.1% formic acid was added to the mobile phase, the peak shape and response value of 3 compounds were obviously improved. Thus, the final determined mobile phase is acetonitrile (0.1% formic acid) - water (0.1% formic acid) at a flow rate of 0.4 mL min⁻¹.

The multi-reaction monitoring (MRM) was used as a quantitative determination method. At the beginning of the experiment, both the positive ion and the negative ion detection modes were investigated. It was found that when the negative ion model was used, the response value of the [M-H]⁻ molecular ion peak was better and the production of molecular ion peak was more stable. In the positive ion mode, the production of ions of 3 compounds are almost undesired and very unstable, so the negative ion detection is used for analysis.

Selection of internal standard

Choosing an appropriate internal standard is of great importance for the analysis of the whole biological samples. During the selection of internal standard, we selected butylparaben, puerarin and esculetin for analysis and investigation. The results showed that the recovery rates of butylparaben and puerarin were so unstable and low that could hardly be detected. It was speculated that hydrochloric acid and sodium hydroxide used in plasma sample treatment reacted with the two compounds, which had an effect on the detection results. Among all the candidate compounds, esculetin showed good response and stable recovery rate with no direct interference with all the compounds. Thus, esculetin was chosen as the internal standard.

Method validation

Selectivity. The blank plasma, blank plasma with reference (LLOQ) and internal standard, quality control sample and internal standard, and chromatograms of 1.5 h after oral extractive of A. euchroma were found in Fig. 2. The retention times of shikonin, isobutyryl shikonin and β, β’-dimethylacryl alkanin were 1.39, 2.98, 3.28 and 0.65 min, respectively. It was observed that the endogenous substances had no obvious interference with the detection of 3 compounds and internal standard.

Linearity and LLOQ. For linearity, the linear regression equation, linear correlation parameters, linear range and LLOQ of shikonin, isobutyryl shikonin and β, β’-dimethylacryl

Fig. 2. MRM chromatograms of shikonin (1), isobutyryl shikonin (2), β, β’-dimethylacryl alkanin (3) and IS (4). Blank plasma (a), blank plasma sample spiked with three analytes at the LLOQ and IS (b), blank plasma sample spiked with three analytes at HQC and IS (c), beagle dog plasma samples collected 1.5 h after oral administration of the extract of A. euchroma (d)
alkalin acanin were observed from Table 2. The linear correlation coefficients of all analytes are greater than 0.99950, indicating that the three analytes have good linear relations in the linear range.

**Precision and accuracy.** About accuracy and precision, Table 3 had shown the accuracy and precision of shikonin, isobutyryl shikonin and β, β'-dimethylacryl alkanin. Intra-day precision and inter-day precision were less than 11.0% and

Table 2. Regression equations, correlation coefficients linear ranges and LLOQs for analytes in beagle dog plasma

| Analytes                       | Equation               | $r$       | Linear range (ng mL$^{-1}$) | LLOQ (ng mL$^{-1}$) |
|--------------------------------|------------------------|-----------|----------------------------|---------------------|
| shikonin                       | $Y=6.50 \times 10^{-3}X-4.1 \times 10^{-3}$ | 0.9965    | 4.060–519.0                | 4.060               |
| isobutyryl shikonin            | $Y=1.73 \times 10^{-3}X-8.3 \times 10^{-3}$ | 0.9983    | 3.180–403.0                | 3.180               |
| β, β'-dimethylacryl alkanin    | $Y=1.90 \times 10^{-3}X+5.8 \times 10^{-3}$ | 0.9994    | 4.370–561.0                | 4.370               |

Table 3. Precision and accuracy of analytes at LLOQ and QC samples in beagle dog plasma (n = 6)

| Analytes              | Spiked concentration (ng mL$^{-1}$) | Mean ± SD (ng mL$^{-1}$) | Intra-day Precision RSD (%) | Inter-day Precision RSD (%) | Accuracy RE (%) |
|-----------------------|-------------------------------------|--------------------------|----------------------------|----------------------------|-----------------|
| shikonin              | 4.060                               | 1.10 ± 0.39              | 9.4                        | 8.8                        | 5.9              |
|                       | 9.730                               | 8.15 ± 0.21              | 3.7                        | 4.6                        | 2.9              |
|                       | 64.87                               | 65.01 ± 3.54             | 4.5                        | 9.6                        | 1.6              |
|                       | 519.0                               | 520.3 ± 25.63            | 5.8                        | 7.3                        | 4.1              |
| isobutyryl shikonin   | 3.180                               | 1.17 ± 0.28              | 9.0                        | 7.8                        | 4.2              |
|                       | 7.550                               | 6.41 ± 0.35              | 5.4                        | 5.9                        | 2.6              |
|                       | 50.37                               | 52.19 ± 4.70             | 8.7                        | 11                         | 4.4              |
|                       | 322.4                               | 323.6 ± 15.23            | 6.3                        | 8.1                        | 2.8              |
| β, β'-dimethylacryl alkanin | 4.370                            | 1.42 ± 0.19              | 7.1                        | 5.2                        | 7.4              |
|                       | 10.51                               | 8.53 ± 1.20              | 4.7                        | 2.1                        | 5.8              |
|                       | 70.12                               | 71.52 ± 4.16             | 6.1                        | 3.5                        | 2.2              |
|                       | 448.8                               | 449.0 ± 23.82            | 5.3                        | 6.5                        | 1.7              |

Table 4. Extraction recovery and matrix effect of analytes in beagle dog plasma (n = 6)

| Analytes              | Spiked concentration (ng mL$^{-1}$) | Extraction recovery | Matrix effect |
|-----------------------|-------------------------------------|---------------------|---------------|
|                       |                                     | Mean (%)            | RSD (%)       | Mean (%)            | RSD (%)       |
| shikonin              | 9.730                               | 89.31 ± 4.75        | 5.32          | 92.30 ± 3.13        | 3.39          |
|                       | 64.87                               | 83.62 ± 8.01        | 9.58          | 97.26 ± 5.92        | 6.09          |
|                       | 415.2                               | 81.46 ± 5.76        | 7.07          | 95.45 ± 4.20        | 4.40          |
| isobutyryl shikonin   | 7.550                               | 85.27 ± 5.47        | 6.41          | 98.83 ± 5.65        | 5.63          |
|                       | 50.37                               | 81.71 ± 10.43       | 12.8          | 94.32 ± 8.39        | 8.89          |
|                       | 322.4                               | 93.38 ± 7.14        | 7.65          | 99.02 ± 5.21        | 5.26          |
| β, β'-dimethylacryl alkanin | 10.51                             | 84.39 ± 5.31        | 6.29          | 101.43 ± 3.65       | 3.60          |
|                       | 70.12                               | 88.47 ± 5.08        | 5.74          | 99.92 ± 6.27        | 6.28          |
|                       | 448.8                               | 82.54 ± 4.03        | 4.88          | 96.65 ± 3.91        | 4.05          |
| IS                   | 250.0                               | 97.37 ± 3.07        | 3.15          | 101.28 ± 4.14       | 4.09          |

Table 5. Stability of analytes in beagle dog plasma under various conditions

| Analytes              | Spiked concentration (ng mL$^{-1}$) | Stability (RE %) | Long-term | Short-term | Three Freeze-thaw | Post-treparation |
|-----------------------|-------------------------------------|-----------------|-----------|------------|-------------------|------------------|
| shikonin              | 9.730                               | –2.29           | 6.31      | 8.07       | 13.1              | –2.29            |
|                       | 64.87                               | –11.0           | –3.75     | 6.83       | 4.92              | –11.0            |
|                       | 415.2                               | 9.67            | 8.45      | 3.71       | –2.59             | 9.67             |
| isobutyryl shikonin   | 7.550                               | 6.05            | 4.77      | 4.58       | 3.47              | 6.05             |
|                       | 50.37                               | –3.13           | 5.38      | 3.11       | 7.16              | –3.13            |
|                       | 322.4                               | 6.88            | 10.1      | 3.52       | 8.89              | 6.88             |
| β, β'-dimethylacryl alkanin | 10.51                             | –3.65           | 6.07      | –4.95      | 7.86              | –3.65            |
|                       | 70.12                               | 5.49            | 3.22      | 8.18       | 8.05              | 5.49             |
|                       | 448.8                               | –3.05           | –1.07     | 5.20       | 6.43              | –3.05            |
the accuracy is in the range of 7.4–5.9%. The experimental results are within an acceptable range, which indicates that the analytical method has good reproducibility.

Recovery and matrix effect. About recovery and matrix effect: The recovery and matrix effect of shikonin, isobutyryl shikonin and \( \beta, \beta'- \)dimethylacryl alkanin were found in Table 4. The results showed that the recovery of the analyte was in the range of 87.71%-93.38% and RSD was less than 12.76% at low, medium and high concentrations, which indicated that the plasma sample treatment method was suitable for the detection of the analyte. The matrix effect is in the range of 92.30%-101.43%, RSD is less than 8.89%. The recovery and matrix effect of esculetin were 97.37% and 101.28%, respectively.

Stability experiments. The stability of shikonin, isobutyryl shikonin and \( \beta, \beta'- \)dimethylacryl alkanin in different environments is shown in Table 5. The RE values of the three analytes under four different storage conditions are in the range of 10.95-13.10%. The results show that under the above conditions, the stability of the analytes is good and has little effect in the analysis process.

Application of the method to pharmacokinetic studies

The UPLC-MS/MS method was applied to the pharmacokinetic study after oral administration of \( A. \) euchroma extract at a dose of 267.1 mg kg\(^{-1}\) of the \( A. \) euchroma extract. The results of pharmacokinetics parameters of three kinds of analytical materials are shown in Table 6, the concentration-time curve is shown in Fig. 3. The results showed that compared with shikonin and isobutyryl shikonin, the absorption and elimination rate of \( \beta, \beta'- \)dimethylacryl alkanin in beagle dogs was faster, the absorption of shikonin was the slowest and the elimination rate of isobutyryl shikonin in beagle dogs was the slowest.

Discussion

Because of the well-developed blood circulation and stable physique, beagle dogs has become an ideal experimental object. There was other method applied to determination of shikonin over the past few years. Huang used LC-ESI-MS/MS method to determine the pharmacokinetics of shikonin in rat plasma [29]. The mean value of the maximum blood concentration (\( C_{\text{max}} \)) was 83.6 ng mL\(^{-1}\) at approximately 1.0 min (\( T_{\text{max}} \), the time to reach \( C_{\text{max}} \)) after single intravenous administration at 5 mg kg\(^{-1}\) of shikonin in rats. However, this study is the first to simultaneous determination the pharmacokinetic behavior of three compounds from \( A. \) euchroma extract and evaluate the pharmacokinetic parameters.

| Analytes                      |
|-------------------------------|
| Analytes                      |
| shikonin                      |
| isobutyryl shikonin           |
| \( \beta, \beta'- \)dimethylacryl alkanin |
| \( C_{\text{max}} \) (ng mL\(^{-1}\)) | 182.36 ± 15.12 | 147.96 ± 16.65 | 197.33 ± 15.42 |
| \( T_{\text{max}} \) (h)      | 1.52 ± 0.19     | 1.42 ± 0.16     | 1.16 ± 0.19     |
| \( T_{1/2} \) (h)             | 3.03 ± 0.31     | 3.66 ± 0.94     | 2.06 ± 0.18     |
| \( AUC_{0-\text{t}} \) (ng h\(^{-1}\) L\(^{-1}\)) | 473.38 ± 55.77 | 249.58 ± 38.27 | 506.68 ± 33.18 |
| \( AUC_{0-\infty} \) (ng h\(^{-1}\) L\(^{-1}\)) | 574.74 ± 60.82 | 292.78 ± 42.24 | 641.08 ± 39.70 |

Table 6. Pharmacokinetic parameters of three analytes in beagle dog plasma after oral administration at 267.1 mg kg\(^{-1}\) of the \( A. \) euchroma extract

Fig. 3. Mean plasma concentration-time profiles of shikonin, isobutyryl shikonin, \( \beta, \beta'- \)dimethylacryl alkanin in beagle dog plasma after oral administration of the extract of \( A. \) euchroma extract.
parameters of isobutyril shikonin and $\beta$, $\beta'$-dimethylacryl alkanin in beagle dogs by UPLC-MS/MS. The difference between this study and the above study is due to the different sample extraction, drugs administration, determination method and so on.

CONCLUSION

In this paper, a rapid, sensitive and selective UPLC-ESI-MS/MS method was successfully applied for the pharmacokinetic study of shikonin, isobutyril shikonin and $\beta$, $\beta'$-dimethylacryl alkanin in beagle dogs plasma after oral administration of A. euchroma extract for 267.1 mg kg$^{-1}$ and the pharmacokinetics of isobutyril shikonin and $\beta$, $\beta'$-dimethylacryl alkanin were firstly detected. These results of the validation are within acceptable limits. Furthermore, the pharmacokinetic results provided relevant data and theoretical basis for clinical research and application of A. euchroma extract.

Conflict of interest: None declared.

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