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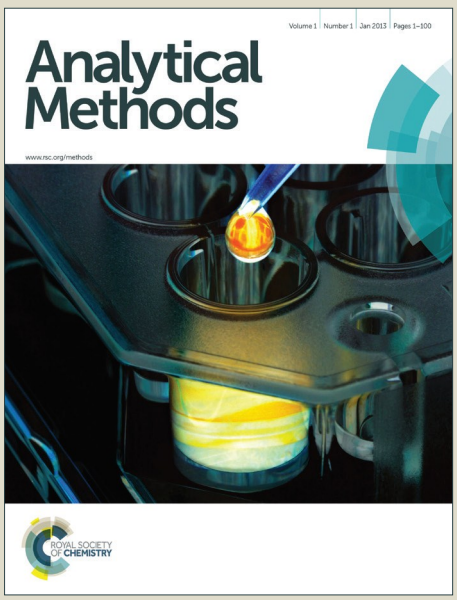

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PAPER

The classification and identification of complex chemical compositions based on UPLC-Q-TOF/MS using yanhusuo herb as an example

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Abstract

The *yanhusuo* herb used in our study is derived from the *yanhusuo* 's dried tubers, which belong to the *Poppy Corydalis* genus, and it is one of the traditional medicinals for relieving pain and promoting the circulation of blood and qi. The main components of *yanhusuo* include tetrahydroprotoberberine alkaloids, protoberberine alkaloids, protopine alkaloids and aporphine alkaloids. In this study, we aimed to determine the classification and identification of the alkaloid components in *yanhusuo* herb by characteristic fragments and neutral losses based on UPLC-Q-TOF/MS technology. After extensive review of the literatures and some reference experiments, we found the fragmentation pattern of several alkaloids and the information of their corresponding fragment ions. Then, we determined the type of compound according to the type of fragment ions and the fragmentation pattern; thus identifying the compounds. Finally, we obtained 20 types of alkaloid compositions, including 12 types of tetrahydroprotoberberine alkaloids, 4 types of protoberberine alkaloids, 2 types of protopine alkaloids and 2 types of aporphine alkaloids. In addition, analysis of the ingredients of *yanhusuo* was performed, which effectively solved the technical difficulties in the fingerprint analysis of Traditional Chinese Medicine (TCM). This accurate and reliable method can provide a foundation for controlling the quality of different batches of the original ingredients.

1 Introduction

Yanhusuo is a sort of Traditional Chinese Medicine (TCM) for relieving pain by promoting the circulation of blood and qi. It was derived from the dried tubers of the *Poppy Corydalis* genus and is commonly used in clinical applications to treat qizhiweitong, amenorrhea dysmenorrhea, postpartum blood stasis and other diseases.[1-4] The main components in *yanhusuo* include protoberberine alkaloids, tetrahydroprotoberberine alkaloids, protopine alkaloids and aporphine alkaloids.[5-15] Today, liquid chromatography-mass spectrometry has been widely used in the ingredients study of TCMs because of its high separation ability, high selectivity and superior sensitivity. Furthermore, UPLC-Q-TOF/MS technology offers the characteristics fast speed, high sensitivity and strong anti-interference ability.[21-25] Therefore, in the process of *yanhusuo* analysis, we scanned the entire spectrum of drugs by the UPLC-Q-TOF/MS technique. However, this method has some drawbacks, including complex spectra and large amounts of information, which lead to some difficulties in the classification and identification of substances. Thus, a different data processing technology need to be developed .

The rapid development of mathematical statistics and chemometrics has developed many new data processing technologies, such as characteristic fragment and neutral loss, which exhibit unique advantages in identifying compounds. Additionally, they are widely used in qualitative and quantitative analyses of substances, metabolomics and marker discovery.[22-33] Characteristic fragments indicate that the compounds of the same or similar public skeleton can be cleaved into different fragments under the energy bombardment in MS, some of which can be used to infer the type of cleavage and the classification of substance, which help to screen target components and filter congeners.[25-28] Otherwise, the molecular ion in the MS can lose neutral radicals or molecules, which are reflected by the discrepancy of m/z between the parent ion and the fragment ions in high mass-to-charge ratio portions. These free radicals or molecules which is known as neutral losses are important in identifying compounds. Thus, in the process of compound identification, by combining the characteristic fragments with the relative neutral losses in MS, the classification and identification of complex ingredients could be determined.[29-33]

The complexity of compounds in TCMs result in great difficulties regarding dose selection in clinical application and preparation production in quality control, which not only hinder their widespread application of TCMs in clinical medicine but also delay their internationalization process. Therefore, the realization of the classification and identification in complex TCMs become very important. In our study, after extensive review of the literatures and some reference experiments, we found the fragmentation pattern and the corresponding fragment information of several alkaloids (protoberberine alkaloids, tetrahydroprotoberberine alkaloids, protopine alkaloids and

aporphine alkaloids). Then, according to the type of fragment ions and the fragmentation patterns, the type of compound could be determined. Finally, combined with the information of neutral losses and its moleculer ion, the compound was identified. In this study, we collected the information on the ingredients in *yanhusuo* herb and their corresponding fragments. Then, by using UPLC-Q-TOF/MS, several alkaloids could be classified and identified. To some extent, this study solved the key problem in identifying the complex chemical compositions of TCMs and had a powerful impact on the rapid development of Chinese medicine identification. In addition, this study also provided a basis for the clinical application of TCMs and the study of pharmacology and metabolomics.

2 Experimental

2.1 Standards and Reagents

Tetrahydropalmatine (AB034R), tetrahydroberberine (AW098T), tetrahydrojatrorrhizine (AR007C), protopine (AW022P), dehydrocorydaline (AW027D), jatrorrhizine (AW101J), allocryptopine (AB015A) and palmatine (AW230P) were obtained from Yifang Technologies, LLC (Tianjin, China). Corydaline (20130815) and coptisine (20130713) were purchased from Shilan Technologies, LLC (Tianjin, China). HPLC-grade acetonitrile, formic acid, methanol and 95% ethanol of analytical grade were purchased from Kangkede Technologies LLC (Tianjin, China). Distilled water was provided by Wahaha Company (Hangzhou, China). The plant *yanhusuo* was purchased from Heyanling Herbal Company (Beijing, China) and identified as the tubers of the *Poppy Corydalis* genus plant *yanhusuo* by pharmacognosy experts.

2. 2 Preparation of standard solution

Precisely weighed tetrahydropalmatine, tetrahydroberberine, tetrahydrojatrorrhizine, protopine, dehydrocorydaline, jatrorrhizine, allocryptopine, palmatine, corydaline and coptisine standards (1 mg each) and placed them into a 25 ml volumetric flask, then filled the mark with 50% methanol solution and shaken. Afterward, the solution was filtered through a 0.22 μm membrane before injection.

2. 3 Preparation of samples

Dried *yanhusuo* medicinal powder (10 g) was added to 10 times and then 8 times the amount of 70% ethanol, which was refluxed for 60 minutes each time and filtered. The two filtrates were then combined and concentrated to 1 g/ml as reserve liquid. Before injection, the sample was diluted to 0.01g/ml, and the sample volume was 5ul.

2. 4 UPLC-Q-TOF/MS conditions

A Waters Acquity UPLC Class I series equipped with a quat pump, an autosampler, and a column compartment was used during analysis. The analytical column was a Waters ACQUITY UPLC BEH C18 Column (2.1×50 mm, 1.7 μm) with the column temperature maintained at 35 ºC. A mobile phase composed of eluent A (0.05% formic acid in water, v/v) and B (0.05% formic acid in acetonitrile, v/v) with a gradient elution was employed for the separation. The flow rate was set at 0.3 mL/min. The linear gradient of solvent B was as follows: from 15% to 30% (1 min), from 30% to 60% (5min), from 60% to 15% (4min) and maintained at 15% for 2min.

UPLC was coupled to the Q-TOF-MS equipped with electrospray ionization (ESI) in positive and negative ion modes. Ultra-high purity helium (He) was used as the collision gas and high-purity nitrogen (N_2) was used as the nebulizing gas. The range of the data acquisition was 50 to 1000 Da. Other operating parameters were as follows: capillary voltage, 3.0 kV; drying gas temperature, 325 ºC; desolvation gas flow rate, 600 $L \cdot h^{-1}$; and nebulizing gas pressure, 350 psi. The Leu-Enkephalin ions at m/z 556.2771 and 554.2615 were used to calibrate the mass accuracy.

3 Results and discussion

3.1 The establishment of analytical method for the classification and identification of compounds

According to their chemical structures, the alkaloids in *yanhusuo* can be divided into four types: protoberberine alkaloids, tetrahydroprotoberberine alkaloids, protopine alkaloids and aporphine alkaloids. The structures of these alkaloids are very similar and often exist isomeric forms, which make the identification of substance confused and difficult.

In our study, after reviewed the literatures extensively, we found, arranged and summarised the fragmentation pattern and the corresponding fragment information of the four types of alkaloid; then we had some reference experiments to confirm the validity of the fragmentation pattern (the MS' information of three representative standards are shown in Fig.2). Firstly, for example, when the structure of the C-ring is saturated, such as in the components of tetrahydroprotoberberine alkaloids and protopine alkaloids, it is easy to undergo RDA-cleavage with complementary fragment ions in MS; but when the structure of the C-ring is unsaturated, such as in the components of berberines and aporphine alkaloids, RDA-cleavage does not occur, while their cleavage pathway are mainly of the substituents. Secondly, based on the relative abundance of the fragments in MS, we could further determine the components which belong to different type of alkaloids. For example, the C-14 position of protopine alkaloids connects to the oxygen to form a carbonyl group, which easily dehydrates in the MS and forms the strongest fragment ions. In contrast, the strongest fragment ions of tetrahydroprotoberberine alkaloids are directly generated by RDA-cleavage. For protoberberine alkaloids and aporphine alkaloids, because of the impact of the nitrogen-atom and the unsaturated C-ring, the structure is more highly conjugated, resulting in higher relative abundance of fragment ions. Protoberberine alkaloids mainly form the highest relative abundance fragment ions by losing a methyl group of 15 Da, whereas aporphine alkaloids lose a methoxy of 31 Da. Meanwhile, in "Zhang et al., 2011" and "Ding et al., 2007", alkaloids in yanhusuo herb were both divided into tertiary alkaloids and quaternary alkaloids, the MS behavior of these compounds are all consistent with the law of fragmentation pattern in our study.

So, according to the rules of compounds' fragments in MS,

we could first compared the respective types according to their characteristic fragments to reduce interference of isomeric phenomena between different types of alkaloid, then combined with other fragment ions and neutral losses, the compound could be exactly identified. First, according to whether the component exist the way of Retro-Diels-Alder (RDA) cleavage in MS, the type of alkaloid which the compound belonged could be determined. For example, when the structure of the C-ring is saturated, such as in the components of tetrahydroprotoberberine alkaloids and protopine alkaloids, it is easy to undergo RDA-cleavage with complementary fragment ions in MS; but when the structure of the C-ring is unsaturated, such as in the components of berberines and aporphine alkaloids, RDA-cleavage does not occur, while their cleavage pathway are mainly of the substituents. Second, based on the relative abundance of the fragments in MS, we could determine the components belonging to which type of alkaloids. For example, the C-14 position of protopine alkaloids connects to the oxygen to form a carbonyl group, which easily dehydrates in the MS and forms the strongest fragment ions. In contrast, the strongest fragment ions of tetrahydroprotoberberine alkaloids are directly generated by RDA-cleavage. For protoberberine alkaloids and aporphine alkaloids, because of the impact of the nitrogen-atom and the unsaturated C-ring, the structure is more highly conjugated, resulting in higher relative abundance of fragment ions. Protoberberine alkaloids mainly form the highest relative abundance fragment ions by losing a methyl group of 15 Da, whereas aporphine alkaloids lose a methoxy of 31 Da. Finally, according to the parent ion and other fragment ions at the same retention time in the MS, the compounds could be identified.

In this study, we determined the classification and identification of the alkaloid components in *yanhusuo* herb by characteristic fragments and neutral losses based on UPLC-Q-TOF/MS technology, including 12 types of tetrahydroprotoberberine alkaloid, 4 types of protoberberine alkaloid, 2 types of protopine alkaloid and 2 types of aporphine alkaloid. The total ion current (TIC) of *yanhusuo* herb in the positive ion mode is shown in Fig. 1. The specific information of those compounds are shown in Table 1 and the structures of some alkaloids are shown in Fig. 3.

3.2 Identification of the compounds in *yanhusuo* herb

3.2.1 Identification of tetrahydroprotoberberine alkaloids

The basic skeleton of tetrahydroprotoberberine alkaloids is an intermediate tetracyclic ring system connecting xylylene to tetrahydroisoquinolines, also known as a C-ring, which is saturated under RDA-cleavage conditions and can be divided into two complementary ions in MS.

Compound 1 exhibited a retention time of 5.10 and a formula of $C_{21}H_{25}NO_4$. In our experiment, we obtained the fragment ions at *m/z* 356.1853, 341.1600, 192.1024, 177.0779, 165.0915 and 150.0677 (as shown in Table 1). The fragment ion at *m/z* 356.1853$[M+H]^+$ was the molecular ion and the highest abundance fragment ion was at *m/z* 192.1024$[M+H\text{-}C_{10}H_{12}O_2]^+$. We also obtained the complementary fragment ion of m/z 165.0915$[M+H\text{-}C_{11}H_{13}NO_2]^+$, which corroborated with the RDA reaction of the C-ring and typical RDA-cleavage characteristics. Therefore, we could infer that compound 1 belongs to the tetrahydroprotoberberine family. Additionally, we obtained the fragment ions at m/z 341.1600$[M+H\text{-}CH_3]^+$ with high mass-to-charge ratios, which displayed losses of 15 Da ($-CH_3$) from the parent ion, respectively. Additionally, the fragment ions at *m/z* 150.0677$[M+H\text{-}C_{11}H_{13}NO_2\text{-}CH_3]^+$ and 177.0779$[M+H\text{-}C_{10}H_{12}O_2\text{-}CH_3]^+$ exhibited losses of 15 Da ($-CH_3$) based on the fragment ions at *m/z* 165.0915 and 192.1024, respectively. Compared with the rule of fragment ions from literature and standards,[13-18] compound 1 was inferred to be tetrahydropalmatine. The specific fragmentation process of tetrahydropalmatine is shown in Fig. 4 as an example.

Compound 2 had a retention time of 5.66 and a formula of $C_{20}H_{21}NO_4$. In our experiment, we obtained the fragment ions at *m/z* 340.1538, 325.1291, 176.0704, 165.0909 and 135.0432 (as shown in Table 1). The fragment ion at *m/z* 340.1538$[M+H]^+$ was a molecular ion and the highest abundance fragment ion was *m/z* 176.0704$[M+H\text{-}C_{10}H_{12}O_2]^+$. We also obtained the complementary fragment ion of m/z 165$[M+H\text{-}C_{10}H_9NO_2]^+$, which corroborated with the RDA reaction of the C-ring and typical RDA-cleavage characteristics. Therefore, we were able to infer that compound 2 belongs to the tetrahydroprotoberberine family. This compound also presented the fragment ions at m/z 325.1291$[M+H\text{-}CH_3]^+$ at a high mass-to-charge ratio, which displayed losses of 15 Da ($-CH_3$) based on the parent ion, respectively. The fragment ion at *m/z* 135.0432 $[M+H\text{-}C_{10}H_9NO_2\text{-}2CH_3]^+$ exhibited two loss of 15 Da ($-CH_3$) based on the fragment ion at *m/z* 165.0909. Compared with the rule of fragment ions from literature and standards,[5,7-8]compound 2 was determined to be tetrahydroberberine.

According to this method, we also identified corydaline, tetrahydrojatrorrhizine, N-methyltetrahydropalmatine, taxilamine, scoulerine, tetrahydrocolumbamine, N-methylcanadine, corybulbine, isocorybulbine and yanhunine for a total of 12 ingredients.

3.2.2 Identification of protoberberine alkaloids

The C-ring of protoberberine alkaloids is an unsaturated pyridine structure, thus it cannot undergo RDA-cleavage and mainly losses a 15 Da ($-CH_3$) substituent.

Compound 13 had a retention time of 6.92 and a formula of $C_{22}H_{23}NO_4$. In our experiment, we obtained the fragment ions at *m/z* 366.1711, 351.1455, 350.1391 and 336.1230 (as shown in Table 1). The fragment ion at *m/z* 366.1711$[M+H]^+$ was a molecular ion and the highest abundance fragment ion appeared at m/z 350.1391$[M+H\text{-}CH_4]^+$ in a high mass-to-charge ratio, which displayed a loss of 16 Da ($-CH_4$) based on the parent ion. Therefore, we could infer that compound 13 belonged to the protoberberine family. We also obtained a fragment ion at m/z 351.1455$[M+H\text{-}CH_3]^+$ in the high mass-to-charge ratio, which displayed a loss of 15 Da ($-CH_3$) based on the parent ion. The parent ion produced a loss of 30 Da ($-2CH_3$) to form the fragment ion at m/z 336.1230$[M+H\text{-}2CH_3]^+$. Compared with the rule of fragment ions from literature and standards.[14-17] The specific fragmentation process of dehydrocorydaline is shown in Fig. 5 as

an example.

Compound 14 had a retention time of 6.26 and a formula of $C_{21}H_{21}NO_4$. In our experiment, we obtained the fragment ions at *m/z* 352.1542, 337.1291, 336.1227, 322.1069 and 292.0969 (as shown in Table 1). The fragment ion at *m/z* 352.1542$[M+H]^+$ was a molecular ion and the highest abundance fragment ion appeared at m/z 336.1227$[M+H\text{-}CH_4]^+$ in the high mass-to-charge ratio, which displayed a loss of 16 Da ($-CH_4$) based on the parent ion. Therefore, we could infer that compound 14 belonged to the protoberberine family. We also obtained a fragment ion at m/z 337.1291$[M+H\text{-}CH_3]^+$ in a high mass-to-charge ratio, which displayed a loss of 15 Da ($-CH_3$) based on the parent ion. The parent ion produced a successive loss of 15 Da ($-2CH_3$) to form the fragment ion at m/z 322.1069$[M+H\text{-}2CH_3]^+$, followed by further loss of 30 Da ($-2CH_3$) to form the fragment ion at m/z 292.0969$[M+H\text{-}4CH_3]^+$. Compared with the rule of fragment ions from literature and standards,[6-11] compound 14 was determined to be palmatine.

According to this method, we also identified berberine, dehydrocorydaline, palmatine, and dehydrocorybulbine for a total of 4 ingredients.

3.2.3 Identification of protopine alkaloids

Similar to tetrahydroprotoberberine alkaloids, the protopine C-ring structure is saturated and can easily undergo RDA-cleavage to produce complementary fragment ions in the MS. The C14 position of protopine alkaloids is connected to the oxygen to form a carbonyl group, which is easily dehydrated in the MS, forming the strongest fragment ions and thereby distinguishing them from tetrahydroberberines.

Compound 17 had a retention time of 4.53 and a formula of $C_{20}H_{19}NO_5$. In our experiment, we obtained the fragment ions at *m/z* 354.1329, 336.1224, 206.0798, 188.0706 and 149.0597 (as shown in Table 1). The fragment ion at *m/z* 354.1329$[M+H]^+$ was a molecular ion and the highest abundance fragment ion was *m/z* 188.0706$[M+H\text{-}C_9H_8O_2\text{-}H_2O]^+$. We also obtained the complementary fragment ion of m/z 149.0597$[M+H\text{-}C_{11}H_{11}NO_3]^+$, which correspoitnds to the RDA reaction of the C-ring and typical RDA-cleavage characteristics. Therefore, we could infer that compound 17 belonged to the protopine family. We also obtained the fragment ions of m/z 206.0798$[M+H\text{-}C_9H_8O_2]^+$, which was the prototypical fragment ion of *m/z* 188.0706$[M+H\text{-}C_9H_8O_2\text{-}H_2O]^+$ in the RDA reaction, and m/z 336.1224$[M+H\text{-}H_2O]^+$ wh a high mass-to-charge ratio, which displayed a loss of 18 Da ($-H_2O$) based on the parent ion. Compared with the rule of fragment ions from literature and standards,[7-10] compound 17 was determined to be protopine. The specific fragmentation process of protopine is shown in Fig. 6 as an example.

According to this method, we also identified allocryptopine for a total of 2 protopine ingredients.

3.2.4 Identification of aporphine alkaloids.

The structure of the C-ring in aporphine alkaloids is unsaturated, thus is does not undergo RDA-cleavage. The cleavage method of the substituents is mainly based on substituted fragments, in which the relative highest abundance fragment ion occurs upon loss of a 31 Da methoxy group.

Compound 19 had a retention time of 2.03 and a formula of $C_{19}H_{21}NO_4$. In our experiment, we obtained the fragment ions at *m/z* 328.1554 and 297.1144 (as shown in Table 1). The fragment ion at *m/z* 328.1554$[M+H]^+$ was a molecular ion, which resulted in the fragment ions at m/z 297.1144$[M+H\text{-}OCH_3]^+$, which displayed a loss of 31 Da ($-OCH_3$). Therefore, Compared with the rule of fragment ions from literature,[8] compound 19 was determined to be isoboldine.

According to this method, we also identified oxoglaucine for a total of 2 aporphine ingredients.

After reviewing the literatures and some reference experiments, we determined the fragmentation pattern of several alkaloids (protoberberine alkaloids, tetrahydroprotoberberine alkaloids, protopine alkaloids and aporphine alkaloids) and the information on their corresponding fragment ions. Then, we were able to determine the type of compound based on the type of fragment ions and fragmentation pattern. Finally, combining this information with the neutral losses, the compounds were identified. In this study, we integrated the information gathered on both the ingredients in *yanhusuo* herb and their corresponding fragments. Using UPLC-Q-TOF/MS technology and data processing, we were able to determine the classification and identification of several alkaloids. To some extent, this study solved the key problem of identifying the complex chemical compositions of TCMs and had a powerful impact on the rapid development of identifying TCMs. Therefore, this study provided a basis for the clinical application of Chinese medicine and the study of pharmacology and metabolomics. Finally, we obtained 20 types of alkaloid compositions in *yanhusuo* herb, including 12 types of tetrahydroprotoberberine alkaloids, 4 types of protoberberine alkaloids, 2 types of protopine alkaloids and 2 types of aporphine alkaloids (their clear chemical structures were shown in Fig.3). In this study, the analysis of ingredients of *yanhusuo* provides a foundation for the quality of different batches of the original control ingredients and improves the study of medicinal ingredients. Moreover, this study, which combines characteristic fragments with neutral losses, will aid the discovery of unknown compounds in TCM on the basis of initial substance classifications and the information from different categories. Additionally, this method reduces the burdensome and complex data processing and provides some guidance for classification and identification of other ingredients of TCM.

4 Conclusions

In this study, we determined the classification and identification of *yanhusuo* herb and obtained 20 types of ingredients in *yanhusuo* herb by characteristic fragment and neutral loss analysis based on UPLC-Q-TOF-MS technology. This method solved the technological problems prevalent in the analysis, classification and identification of these compounds caused by their complex compositions. Moreover, this method promotes the development of effective classification and identification of Chinese medicine and provides a new method for screening target compositions.

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Table 1. Retention time, MS data, Error and elemental composition for the target chemical compounds of *yanhusuo* herb screened out by using UPLC-Q-TOF/MS in positive ion mode.

No.	Retention time	Measured mass	Error	Formula	ESI-MS/MS(*m/z*)	Identification	Reference
1	5.10	356.1853	2.53	$C_{21}H_{25}NO_4$	356.1853$[M+H]^+$, 341.1600$[M+H-CH_3]^+$, 192.1024$[M+H-C_{10}H_{12}O_2]^+$, 177.0779$[M+H-C_{10}H_{12}O_2-CH_3]^+$, 165.0915$[M+H-C_{11}H_{13}NO_2]^+$, 150.0677$[M+H-C_{11}H_{13}NO_2-CH_3]^+$	Tetrahydropalmatine	13-18
2	5.66	340.1538	3.23	$C_{20}H_{21}NO_4$	340.1538$[M+H]^+$, 325.1291$[M+H-CH_3]^+$, 176.0704$[M+H-C_{10}H_{12}O_2]^+$, 165.0909$[M+H-C_{10}H_9NO_2]^+$, 135.0432 $[M+H-C_{10}H_9NO_2-2CH_3]^+$	Tetrahydroberberine	5,7-8
3	5.86	370.2010	2.16	$C_{22}H_{27}NO_4$	370.2010$[M+H]^+$, 355.1751$[M+H-CH_3]^+$, 354.1697$[M+H-CH_4]^+$, 340.1543 $[M+H-2CH_3]^+$, 192.1022$[M+H-C_{11}H_{14}O_2]^+$, 179.1063$[M+H-C_{11}H_{13}NO_2]^+$, 177.0786$[M+H-C_{11}H_{14}O_2-CH_3]^+$, 164.0827$[M+H-C_{11}H_{13}NO_2-CH_3]^+$	Corydaline	12-17
4	4.05	342.1704	0.29	$C_{20}H_{23}NO_4$	342.1704$[M+H]^+$, 327.1458$[M+H-CH_3]^+$, 326.1384$[M+H-CH_4]^+$, 312.1565$[M+H-2CH_3]^+$, 178.0867$[M+H-C_{10}H_{12}O_2]^+$, 165.0904$[M+H-C_{10}H_{11}NO_2]^+$, 163.0632$[M+H-C_{10}H_{12}O_2-CH_3]^+$, 135.0680$[M+H-C_{10}H_{11}NO_2-2CH_3]^+$	Tetrahydrojatrorrhizine	17-18
5	6.05	370.2009	2.43	$C_{22}H_{28}NO_4^+$	370.2009$[M+H]^+$, 340.1526$[M+H-2CH_3]^+$, 206.1163$[M+H-C_{10}H_{12}O_2]^+$, 191.0912$[M+H-C_{10}H_{12}O_2-CH_3]^+$, 176.0701$[M+H-C_{10}H_{12}O_2-2CH_3]^+$, 165.0909$[M+H-C_{12}H_{15}NO_2]^+$, 150.0673$[M+H-C_{12}H_{15}NO_2-CH_3]^+$, 135.0709$[M+H-C_{12}H_{15}NO_2-2CH_3]^+$	N-methyltetrahydropalmatine	13,18
6	2.85	328.1552	0.91	$C_{19}H_{21}NO_4$	328.1552$[M+H]^+$, 314.1750$[M+H-CH_2]^+$, 178.0864$[M+H-C_9H_{10}O_2]^+$, 163.0626$[M+H-C_9H_{10}O_2-CH_3]^+$, 151.0753$[M+H-C_{10}H_{11}NO_2]^+$	Scoulerine	20
7	3.86	342.1702	0.87	$C_{20}H_{23}NO_4$	342.1702$[M+H]^+$, 327.1437$[M+H-CH_3]^+$, 326.1387$[M+H-CH_4]^+$, 178.0867$[M+H-C_{10}H_{12}O_2]^+$, 165.0911$[M+H-C_{10}H_{11}NO_2]^+$, 163.0632$[M+H-C_{10}H_{12}O_2-CH_3]^+$	Tetrahydrocolumbamine	13-17
8	4.71	354.1733	7.91	$C_{21}H_{24}NO_4^+$	354.1733$[M+H]^+$, 190.0820$[M+H-C_{10}H_{12}O_2]^+$, 165.0540$[M+H-C_{11}H_{12}NO_2]^+$, 135.0440$[M+H-C_{11}H_{12}NO_2-2CH_3]^+$	N-methylcanadine	10

9	4.01	356.1856	1.68	$C_{21}H_{25}NO_4$	356.1856$[M+H]^+$, 341.1602$[M+H-CH_3]^+$, 340.1541$[M+H-CH_4]^+$, 179.0899$[M+H-C_{10}H_{11}NO_2]^+$, 178.0862$[M+H-C_{11}H_{14}O_2]^+$, 163.0628$[M+H-C_{11}H_{14}O_2-CH_3]^+$	Corybulbine or Isocorybulbine	12-13,17
10	4.18	356.1855	1.97	$C_{21}H_{25}NO_4$	356.1855$[M+H]^+$, 341.1611$[M+H-CH_3]^+$, 326.1375$[M+H-2CH_3]^+$, 179.0912$[M+H-C_{10}H_{11}NO_2]^+$, 178.0858$[M+H-C_{11}H_{14}O_2]^+$, 163.0642$[M+H-C_{11}H_{14}O_2-CH_3]^+$	Isocorybulbine or Corybulbine	12-13,17
11	4.32	356.1845	4.77	$C_{21}H_{25}NO_4$	356.1845$[M+H]^+$, 341.1613$[M+H-CH_3]^+$, 326.1375$[M+H-2CH_3]^+$, 192.1018$[M+H-C_{10}H_{12}O_2]^+$, 177.0785$[M+H-C_{10}H_{12}O_2-CH_3]^+$, 165.0905$[M+H-C_{11}H_{13}NO_2]^+$, 150.0683$[M+H-C_{11}H_{13}NO_2-CH_3]^+$	Yanhunine	12
12	5.20	370.1257	9.18	$C_{20}H_{19}NO_6$	370.1257$[M+H]^+$, 356.1853$[M+H-CH_2]^+$, 192.1018$[M+H-C_{10}H_{12}O_2-CH_2]^+$, 165.0908$[M+H-C_{12}H_{15}NO_2]^+$	Taxilamine	17
13	6.92	366.1711	1.64	$C_{22}H_{24}NO_4^+$	366.1711$[M+H]^+$, 351.1455$[M+H-CH_3]^+$, 350.1391$[M+H-CH_4]^+$, 336.1230$[M+H-2CH_3]^+$	Dehydrocorydaline	14-17
14	6.26	352.1546	0.85	$C_{21}H_{22}NO_4^+$	352.1542$[M+H]^+$, 337.1291$[M+H-CH_3]^+$, 336.1227$[M+H-CH_4]^+$, 322.1069$[M+H-2CH_3]^+$, 292.0969$[M+H-4CH_3]^+$	Palmatine	6-11
15	6.44	352.1533	4.54	$C_{21}H_{22}NO_4^+$	352.1533$[M+H]^+$, 337.1284$[M+H-CH_3]^+$, 336.1225$[M+H-CH_4]^+$, 322.1058$[M+H-2CH_3]^+$, 321.0977$[M+H-OCH_3]^+$	Dehydrocorybulbine	15-17
16	6.38	336.1231	1.49	$C_{20}H_{18}NO_4$+	336.1231$[M+H]^+$, 321.0992$[M+H-CH_3]^+$, 320.0920$[M+H-CH_4]^+$, 306.0759$[M+H-2CH_3]^+$	Berberine	13-18
17	4.53	354.1329	3.39	$C_{20}H_{19}NO_5$	354.1329$[M+H]^+$, 336.1224$[M+H-H_2O]^+$, 206.0798$[M+H-C_9H_8O_2]^+$, 188.0706$[M+H-C_9H_8O_2-H_2O]^+$, 149.0597$[M+H-C_{11}H_{11}NO_3]^+$	Protopine	7-10
18	5.03	370.1656	0.54	$C_{21}H_{23}NO_5$	370.1656$[M+H]^+$, 352.1544$[M+H -H_2O]^+$, 322.1056$[M+H -H_2O-2CH_3]^+$, 206.0815$[M+H-C_{10}H_{12}O_2]^+$, 188.0709$[M+H-C_{10}H_{12}O_2-H_2O]^+$ 165.0915$[M+H-C_{11}H_{11}NO_3]^+$	Allocryptopine	7-8,15
19	2.03	328.1554	1.52	$C_{19}H_{21}NO_4$	328.1554$[M+H]^+$, 297.1144$[M+H-OCH_3]^+$	Isoboldine	8
20	6.3	352.1542	1.99	$C_{20}H_{17}NO_5$	352.1642$[M+H]^+$,	Oxoglaucine	17,19

321.0997$[M+H-OCH_3]^+$

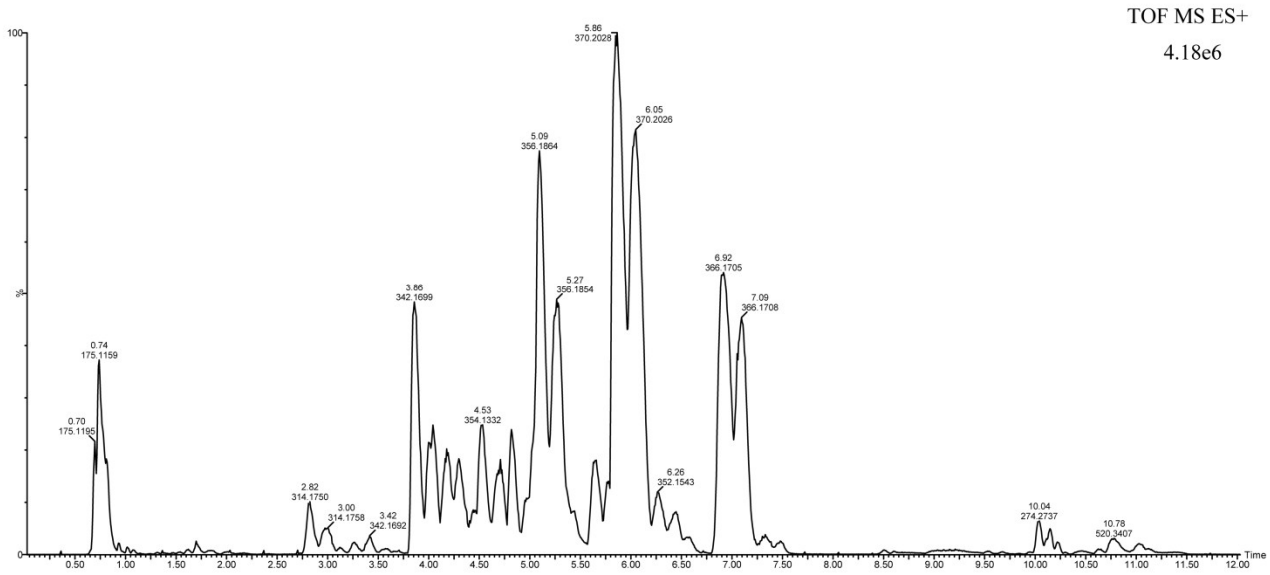


Fig.1 Total ion current (TIC) chromatograms of substances in the extract of *yanhuosuo* herb, under positive ion mode.

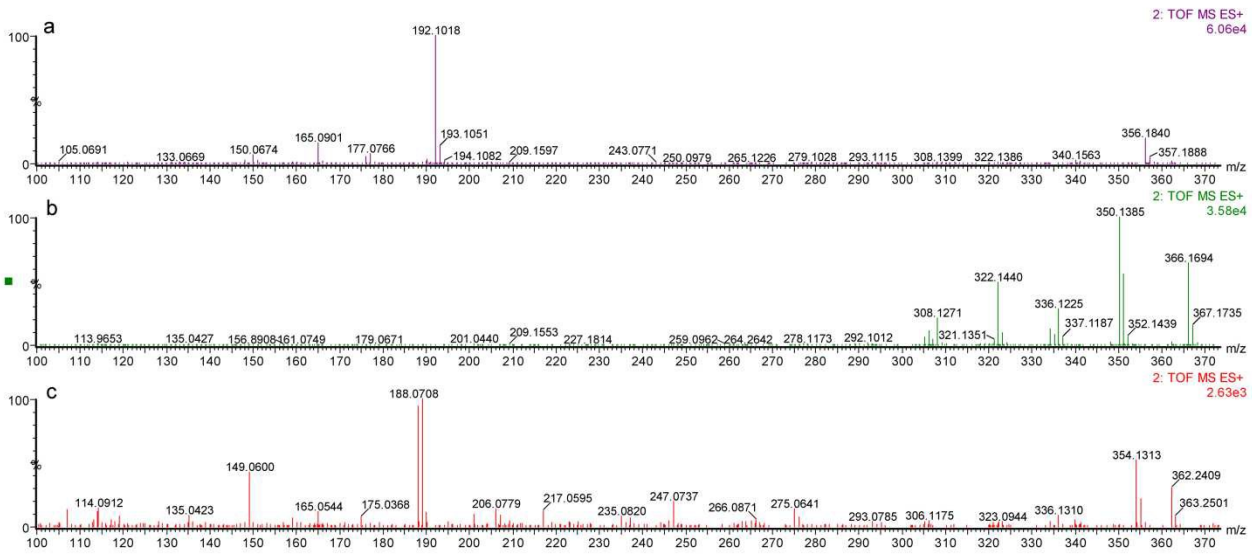
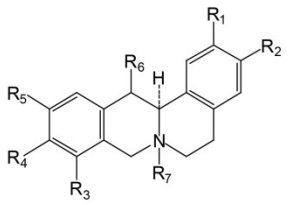


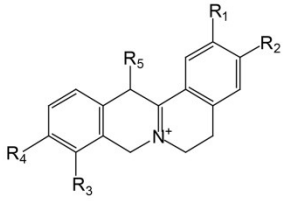
Fig.2 The MS^2 chromatograms for three standards of tetrahydropalmatine (a), dehydrocorydaline (b) and protopine (c).

A



Tetrahydroprotoberberine alkaloids	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Tetrahydropalmatine	OCH_3	OCH_3	H	OCH_3	OCH_3	H	-
Tetrahydroberberine	-O-CH_2-O-		OCH_3	OCH_3	H	H	-
N-methyltetrahydropalmatine	OCH_3	OCH_3	OCH_3	OCH_3	H	H	CH_3
N-methylcanadine	-O-CH_2-O-		OCH_3	OCH_3	H	H	OCH_3

B



Protoberberine alkaloids	R_1	R_2	R_3	R_4	R_5
Dehydrocorydaline	OCH_3	OCH_3	OCH_3	OCH_3	CH_3
Palmatine	OCH_3	OCH_3	OCH_3	OCH_3	H
Dehydrocorybulbine	OCH_3	OH	OCH_3	OCH_3	CH_3
Berberine	-O-CH_2-O		OCH_3	OCH_3	H

C

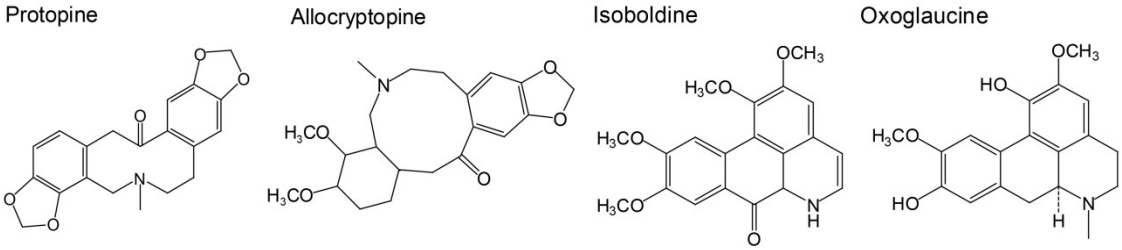


Fig.3 The specific structures' information of some tetrahydroprotoberberine alkaloids were shown in A, the specific structures' information of some protoberberine alkaloids in B and specific structures' information of the compound of protopine alkaloids and aporphine alkaloids in C.

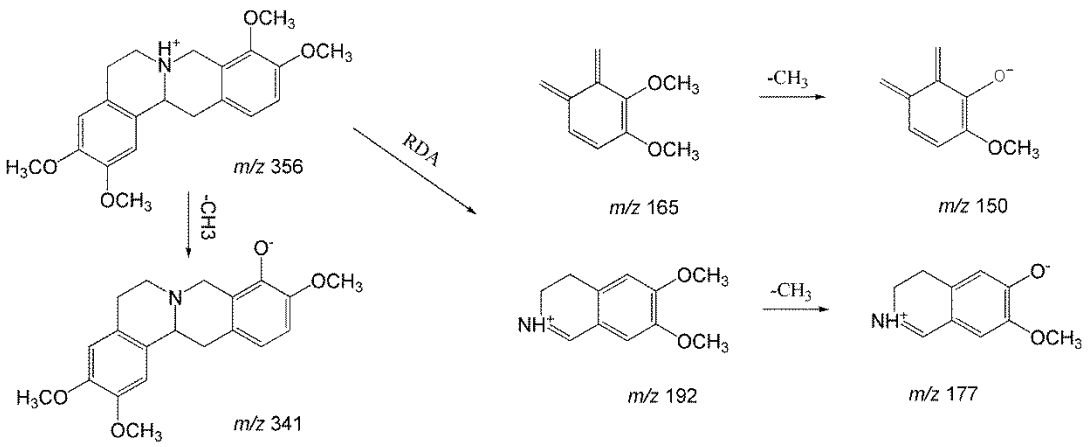


Fig.4 The proposed fragmentation pathway for tetrahydroprotoberberine alkaloids, take tetrahydropalmatine as an example: the fragment ion at *m/z* 356 was the molecular ion $[M+H]^+$ and the fragment ions at *m/z* 192 and m/z 165 were corroborated with the RDA reaction of the C-ring and typical RDA-cleavage characteristics.

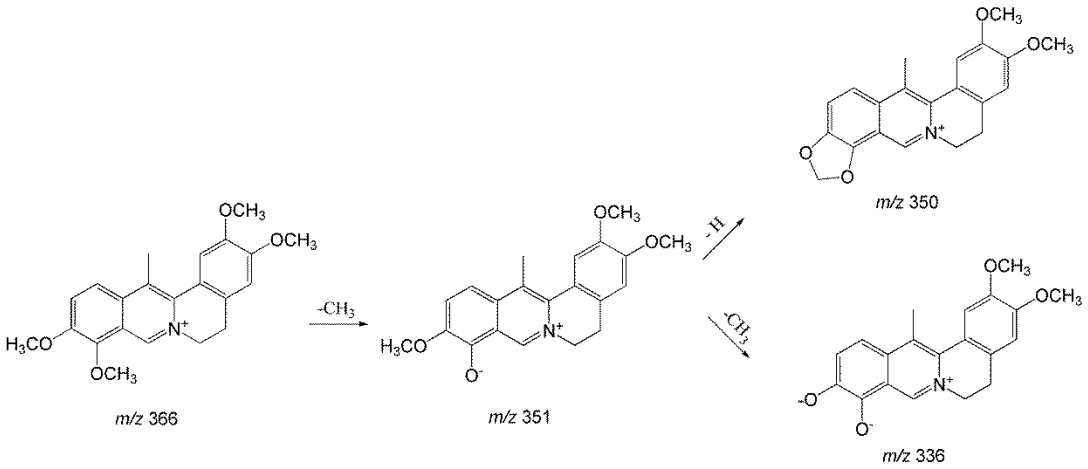


Fig. 5 The proposed fragmentation pathway for protoberberine alkaloids, take dehydrocorydaline as an example: the fragment ion at *m/z* 366 was a molecular ion $[M+H]^+$ and the highest abundance fragment ion appeared at *m/z* 350 and 351 in a high mass-to-charge ratio, which displayed a loss of 16 Da ($-CH_4$) based on the parent ion. Therefore, it own the cleavage characteristic of the protoberberine family.

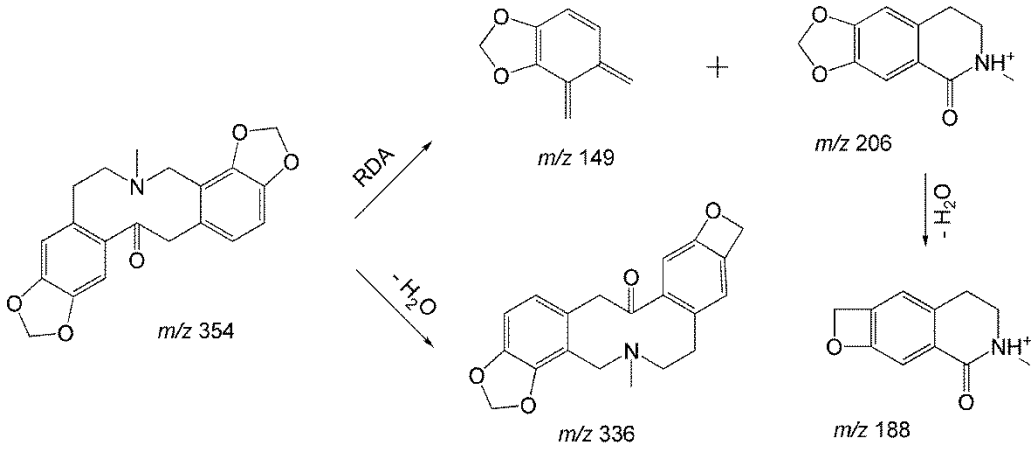
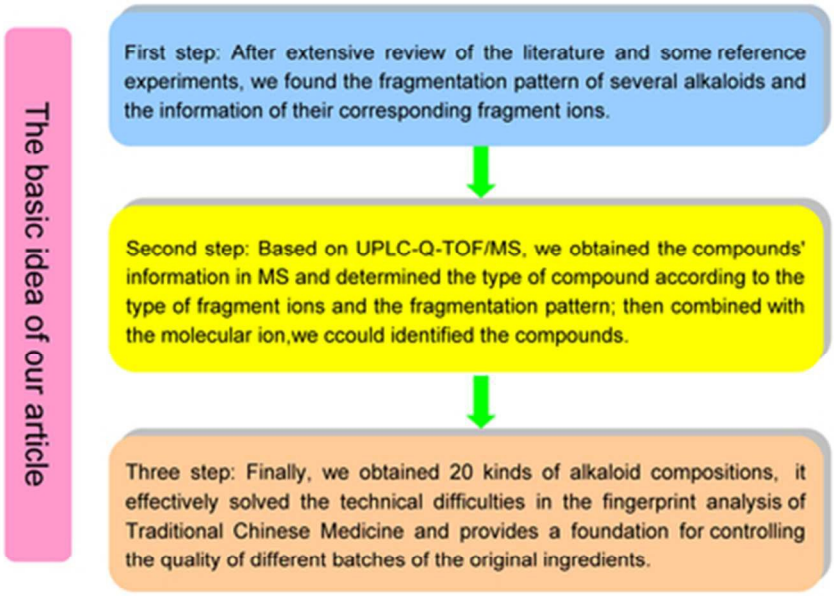


Fig.6 The proposed fragmentation pathway for protopines alkaloids, take protopine as an example: the fragment ion at *m/z* 354 was a molecular ion $[M + H]^+$; the highest abundance fragment ion was *m/z* 188 and its prototypical fragment ion of *m/z* 206; otherwise the complementary fragment ion of m/z 149, which corresponds to the RDA-cleavage characteristics of the protopine family.



A method that applied for the classification and identification of complex chemical compositions in yanhusuo herbs based on UPLC-Q-TOF/MS coupled with characteristic fragment
39x29mm (300 x 300 DPI)