ThP280

Distribution of active pharmaceutical ingredients in Forthysia suspensa and quality control with mass spectrometry imaging

Zhenhe Chen[1], Kaoru Nakagawa[2], Jian Su[3], Keisuke Shima[1], Yongli Liu[3*], Rong Lei[3], Jing Dong[1], Hao Yuan[3], Xiaolei Wang[3], Satoshi Kasamatsu[4], Lei Cao[1], Xiaodong Li*[1]

1. Shimadzu China Innovation Center, Shimadzu Corporation, Beijing, China; 2. Healthcare Solution Unit, Solutions Center of Excellence, Shimadzu Corporation, Kyoto, Japan; 3. Hebei Institute for Drug and Medical Device Control, Shijiazhuang, China. 4. MS Business Unit, Life Science Business Department, Shimadzu Corporation, Kyoto, Japan

1. Introduction

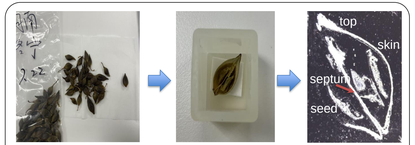
The fruit of Forthysia suspensa (Thunb.) Vahl (*F. suspensa*) is a traditional Chinese medicine (TCM) and is proved to have significant anti-inflammatory, antibacterial, and antiviral activities. Its active pharmaceutical ingredients include lignans, phenylethanol glycosides, flavonoids, organic acids, *etc*. As a major component of 'Lianhua Qingwen', the fruit of *F. suspensa* has been used to treat covid-19 infection and other cold symptoms. Ripe and green fruits of *F. suspensa* have different indications and are both included in China Pharmacopeia (2020). Meanwhile, their contents of active ingredients varied as harvest time, place of production, and cultivation (wild/cultivated). Traditionally, the contents of active ingredients are evaluated by GCMS or LCMS methods. However, the distribution of active ingredients was not known, and mass imaging spectrometry (MSI) has not been used for its quality control.

2. Materials and Methods

Fruits of *F. suspensa* were obtained from several famous habitats from 2019 to 2022 in China (Table 1). According to the Chinese Pharmacopeia (2020 ed.), the fruits of *F. suspensa* are used as medicine after steaming and drying in the sun. MSI was used to investigate the distribution of active ingredients in *F. suspensa* fruit sections. The preparation of the fruit section was performed according to a normal procedure with minor modifications. Primarily, the treated fruit of *F. suspensa* was separated in half according to its texture. Obviously, the fruit mainly includes skin, seeds, and two compartments separated by the septum (Figure 1). Primarily, half of the fruit was directly embedded in 10% gelatin, but the obtained sections were fragile. Alternatively, the seeds were removed carefully and backfilled in the compartments immediately before the gelatin solidified and intact sections were thus obtained. After freezing in a -80 ℃ refrigerator for two hours, the samples were transferred to a microtome (Leica, Germany) for sectioning.

Table1 Habitats and sampling time of *F. suspensa* fruits

Habitat	green fruits			ripe fruits		
	wild	cultivated	sampling time	wild	cultivated	sampling time
Caochuan, Shanxi	3	2	2022.7	2	—	2020.10
Luoning, Henan	1	—	2022.9	—	—	—
Handan, Heibei	2	—	2019.8	1	—	2019.10



F. suspensa fruits Gelatin embedding Fruit section

Figure 1 Sectioning of *F. suspensa* fruit

iMScope QT

The section thickness is 15 μm and optical images were recorded with microscope at 10x objective lens. DHB and 9AA were vapor-deposited on the sample surface using a Shimadzu iMLayer™ matrix vapor deposition system (Shimadzu, Kyoto, Japan) for positive and negative mass mode, respectively. Mass imaging was performed on iMScope QT (Shimadzu, Kyoto, Japan) equipped with an optical microscope, an atmospheric pressure MALDI source (AP-MALDI), and a quadrupole time-of-flight mass spectrometer. Table 2 lists instrument parameters for mass imaging. Statistical analysis and image reconstruction were carried out with IMAGEREVEAL™MS (Shimadzu, Kyoto, Japan).

Table 2 Instrument parameters for mass imaging

Imaging parameters		Mass analysis parameters	
pixel	10 or 20 μm	mass stages	MS or MS/MS
laser diameter setting	1 or 2	heat block	250 ℃
laser shots	100	mass range	*m/z* 100-500, and 500-1000
laser repetition frequency	2 kHz	polarity	+ or -
laser intensity	55-68	detector voltage	2.35 kV

More than 125 active ingredients were reported in *F. suspensa* fruit by a literature review. Their theoretical mass values were collected and summarized as a screening list for data analysis. Table 3 listed detected compounds with mass imaging spectrometer in positive and negative mass mode, respectively. For the comparison of different samples, the distribution of 8 major active ingredients was compared in three fruit subsections (skin, seed, and top). The obtained images were scored according to the intensity and distribution of each active ingredient, and the sum of scores of 8 ingredients was used as an indicator of a better *F. suspensa* fruit.

Table 3 Active ingredients detected with mass imaging

Polarity	*m/z* 100-500	*m/z* 500-1000	Total
Positive (+)	>27	>16	>43
Negative (-)	>22	>6	>28

3. Results

3-1. Active ingredients detected with AP-MALDI mass imaging

In this study, an AP-MALDI mass imaging spectrometer was applied for the detection of active ingredients. With the screening list, it is found that more than 43 and 28 active ingredients were detected (Table 3). For instance, forsythin and forsythoside A are two major active ingredients in *F. suspensa* and they are used to evaluate the quality of *F. suspensa* according to Chinese Pharmacopeia. More than 18 standards were obtained to confirm the detected compounds. For instance, *m/z* 623.19857 was confirmed as forsythoside A because they had the same precursor and product ion (Figure 4). Forsythoside A can be detected in both positive and negative mode but forsythin can only be detected in positive mode (Figure 2 and Figure 3).

It is found that the active ingredients were not homogeneously distributed in the fruit top (Figure 3) and it is the same in the negative mode (Figure 3). Especially, oleanic acid and/or ursolic acid were only distributed in the external part of the fruit skin. In contrast, suspensaside A distributed homogeneously in the fruit skin. Except for known active ingredients, unknown compounds could also be found in the fruits (Figure 5). Most of them were distributed in the skin of seeds and some of them were mainly distributed in seeds (Figure 5). However, these compounds were not identified.

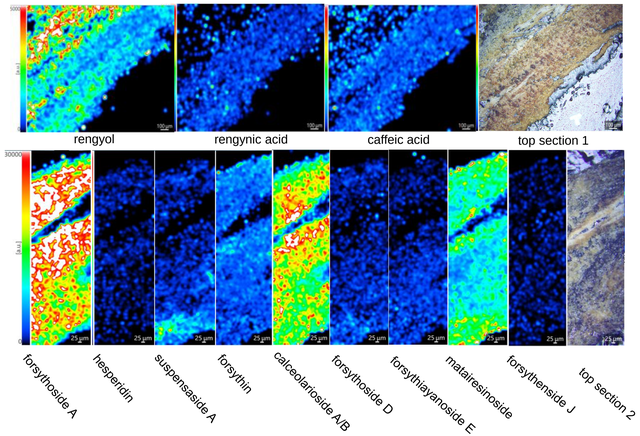


Figure 2 Active ingredients detected in positive ion mode

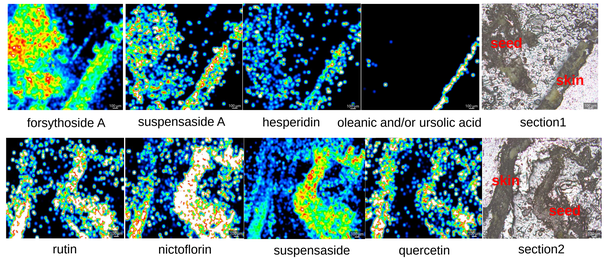


Figure 3 Active ingredients detected in negative ion mode

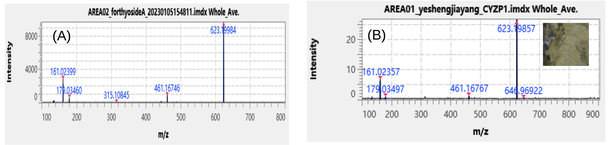


Figure 4 Product scan spectra of fortythoside A standard (A) and *m/z* 623.19857 in the real sample (B，inset is a picture of fruit top)

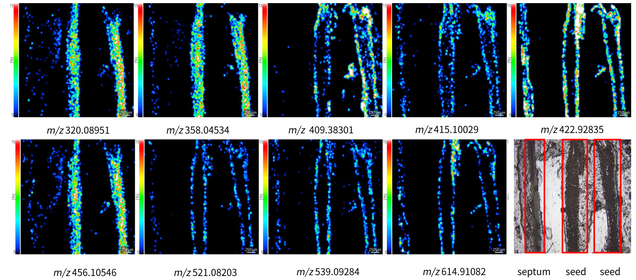


Figure 5 Detected compounds not identified in *F. suspensa* fruit

3-2. Quality control of *F. suspensa* fruit

For a long time, the quality control of *F. suspensa* fruit has been a hot topic. Traditionally, the contents of forsythin and forsythoside, volatile oil, and extracts were used to evaluate the quality of *F. suspensa* fruit. Consequently, it is hard to correlate each active ingredient with pharmaceutical potency. LCMS or GCMS were able to test each ingredient, but the pretreatment and analysis of samples are complicated. In comparison, MSI could be used to evaluate as many as 43 active ingredients of *F. suspensa* fruit in one analysis in very short time.

Eight major components were used to evaluate different samples (salidroside, cornoside, rengyoside A, adoxosidic acid, calceolarioside A/B, epipinoresinol-4-O-β-D-glucoside, forsythin, forsythoside A). As a result, the green fruit is better than the ripe one from Caochuan. But it is inverse for fruits from Handan and two replicates showed the same results (Table 4). It is inconsistent with common sense that the green fruit has more active ingredients than the ripe one. Harvest time is another factor that affects the quality of fruit. For both *F. suspensa* fruits from Luoning and Caochuan, the best harvest time should be before the end of July. The early harvest time would benefit the contents of active ingredients (Table 4). Meanwhile, the active ingredients vary differently from different batches and habitats (data not shown here) and MSI could be a reliable and efficient way to fully evaluate *F.suspensa* fruits.

Table 4 The quality comparison of *F. suspensa* fruits

type	habitat	subsections, replicates	result
ripe *vs.* green	Handan	skin, seed, end, n=2	ripe > green
	Caochuan	skin, seed, end, n=1	green > ripe
harvest time (green)	Luoning	skin, seed, end, n=1	7.25 > 8.9 > 8.22
	Caochuan	skin, seed, end, n=1	7.28 > 8.14

4. Conclusions

- More than 43 and 28 active pharmaceutical ingredients could be detected in *F.suspensa* fruits with AP-MALDI imaging in positive and negative ion mode, respectively.
- As a choice, MSI could be used to evaluate the quality of the *F.suspensa* fruit fast and rationally.