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Chemotype Discrimination and Rapid Identification of Angelica Roots by DART-TOF-MS†

Hye Jin Kim[1], Xiang-lan Piao[2], and Young Pyo Jang[1,3,*]

[1]*Division of Pharmacognosy, College of Pharmacy, Kyung Hee University, Hoegi-dong, Dongdaemun-gu, Seoul, 130-701, Republic of Korea*
[2]*Institute of Chinese Minority Traditional Medicine, Minzu University of China, Beijing 100081, China*
[3]*Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130-701, Republic of Korea*

Abstract – The Angelica root has been used as a medicinal herb in many Asian countries including Korea, China, and Japan. *Angelica gigas, A. sinensis*, and *A. acutiloba* have been considered as Angelicae radix in Korean, Chinese, and Japanese Pharmacopoeia, respectively. Since the origins of Angelicae radix differ from country to country, there is a need to develop an efficient analytical method to identify the origin of the Angelica root. In order to obtain chemical fingerprints, three different Angelicae Radices were analyzed by direct analysis in real time mass spectrometry (DART-MS). Significantly different DART-MS spectra were observed from three different species of Angelicae Radix. Strong peaks of decursin or decusinol angelate, and its dimer were exclusively found from *A. gigas*. Ligustilide and linoleic acid were detected as the major component from *A. acutiloba*. The strongest ligustilide peak was observed from *A. sinensis*. DART-MS fingerprinting is a promising method for the rapid identification and/or quality control of Angelicae Radix.
Keywords – DART-TOF-MS (Direct analysis in real time - Time of flight - Mass spectrometry), *Angelica gigas, Angelica acutiloba, Angelica sinensis*

Introduction

Angelicae Radix has been widely used in Korea, Japan, and China for the treatment of a wide variety of gynecological diseases such as anemia and menstrual disorders (Hatano *et al.*, 2004, Hou *et al.*, 2005, Liu *et al.*, 2001, Yook, 1990). Different origins of Angelicae Radix have been described in Korean, Japanese and Chinese Pharmacopeia: *Angelica gigas*, *A. acutiloba*, and *A. sinensis*. Since each species contains different phytochemical constituents, there is a need to establish an efficient discrimination method for the quality control of related medicinal products. Previous studies have analyzed the three species of Angelica using HPLC/UV and GC-MS (Lu *et al.*, 2005, Piao *et al.*, 2007, Zhao *et al.*, 2003).

As an ambient ionization technique such as, desorption electrospray ionization (DESI) (Takats *et al.*, 2004) and atmospheric solids analysis probe (ASAP) (McEwen *et al.*, 2005), Direct analysis in real time (DART) ion source (Cody *et al.*, 2005) can also ionize compounds from the intact state of samples. Previous successful applications of DART-MS for crude herbal drug analysis showed that this method could be utilized for the efficient analysis of biologically important compounds *in situ* (Kim and Jang, 2009, Madhusudanan *et al.*, 2008).

In this study, we used DART-TOF-MS for the fast discrimination of three related Angelica root species (*Angelica gigas, A. acutiloba*, and *A. sinensis*) by comparing chemical fingerprints.

Experimental

Materials – Radix part of *Angelica gigas* Nakai. and *Angelica acutiloba* (Sieb. & Zuc.) Kitagawa were obtained from a domestic Korean market (Kyungdong Crude Drugs Market, Seoul, South Korea). *Angelica sinensis* (Oliv.) Diels was purchased at a Chinese market. These medicinal drugs were identified by one of the authors (YPJ) and Chang Soo Yook (Kyung Hee University). Voucher specimens (KHUP-0107, KHUP-0108, KHUP-0109) were deposited in the Museum of Korean Crude Drugs located at College of Pharmacy, Kyung Hee University. For precise measurements using

†Dedicated to Prof. Young Choong Kim of the Seoul National University for her leading works on Pharmacognosy
*Author for correspondence
Tel: +82-2-962-9421; E-mail: ypjang@khu.ac.kr

direct analysis in real time-time of light-mass spectrometry (DART-TOF-MS), each sample was ground to a fine powder.

DART-TOF-MS analysis – The mass spectrometer was an AccuTOF-TLC single-reflectron time-of-flight mass spectrometer (JEOL, Tokyo, Japan) equipped with DART ion source (IonSense, Saugus, MA) and operated with Mass center version 1.3.7 software. In the positive ion mode, the atmospheric pressure interface potentials were set to the following values: orifice 1 = 15 V, ring lens and orifice 2 = 5 V. The rf ion guide potential and detector voltage were set to 500 V and 2200 V. DART electrode potentials were set to needle (glow discharge) electrode = 3200 V, electrode 1 = 100 V, electrode 2 (grid) = 250 V. The gas temperature was set to 250 °C, and gas flow rate was 2.5 liters per minute. Polyethylene glycol 600 (PEG 600) was used as an external reference standard for exact mass measurements. Using a 10 μL glass capillary (Paul Marienfold GmbH, Germany), 2~3 mg of each powder was packed and then directly introduced into the ion source. The analysis time was less than 1 min per sample. Triplicate measurements for each sample were averaged to acquire more accurate and consistent mass spectra.

Results and discussion

Intact Angelica raw materials were readily analyzed by placing them in front of the DART ion source for a few seconds without any previous sample preparation. Representative DART-MS spectra of three Angelica roots are shown in Fig. 1. The spectra were successfully discriminated into 3 different species according to their mass spectra patterns. Elemental compositions of the major peaks were calculated using built-in software based on the exact mass numbers of the elements. One of the major peaks, which had a mass to charge ratio of 329.1407, was annotated as decursin or decursinol angelate from Korean Angelica (Fig. 2). Since these two compounds have the same molecular formula, a clear distinction between them was not possible for this kind of mass spectrometric analysis. The ion peak at *m/z* 657.2705 can be attributed to a dimer of decursin or decursinol angelate (Fig. 2). Protonated dimers were observed in the DART-TOF-MS, especially for some compounds at high concentration (Petucci *et al.*, 2007). The calculated errors for the empirical mass numbers for decursin/decursinol angelate and the dimer form of the peak were less than 2 mmu as compared with their theoretical mass numbers (Table 1). The high resolution power of TOF-MS allowed for efficient confirmation of the detected molecular ions by comparing the measured molecular mass with the corresponding theoretical molecular mass. The major components detected in *A. gigas* by DART-TOF-MS were consistent with previous studies (Piao *et al.*, 2007).

In the mass spectrum from the *A. acutiloba* sample, ligustilide and linoleic acid were determined to be the major peaks (Fig. 1(B)). Previous analyses have demonstrated that the main component from the radix of *A. acutiloba* was ligustilide by HPLC-UV and GC-MS (Piao *et al.*, 2007, Lu *et al.*, 2005, Zhao *et al.*, 2003). Linoleic acid was detected from volatile oil of this plant by GC-

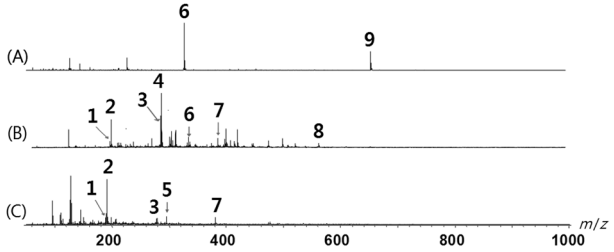

Fig. 1. Comparison of the total ion spectrum from three different Angelicae Radix. (A) *Angelica gigas* (B) *Angelica acutiloba* (C) *Angelica sinensis.*

1 $C_{12}H_{12}O_2$ (butylidenephthalide), **2** $C_{12}H_{14}O_2$ (ligustilide), **3** $C_{16}H_{22}O_4$ (dibutyl phthalate), **4** $C_{18}H_{32}O_2$ (linoleic acid), **5** $C_{19}H_{34}O_2$ (octadecadienoic acid, methyl ester), **6** $C_{19}H_{20}O_5$ (decursin or decursinol angelate), **7** $C_{24}H_{28}O_4$ (ligustilide dimer), **8** $C_{36}H_{64}O_4$ (linoleic acid dimer), **9** $C_{38}H_{40}O_{10}$ (dimer form of decursin or decursinol angelate)

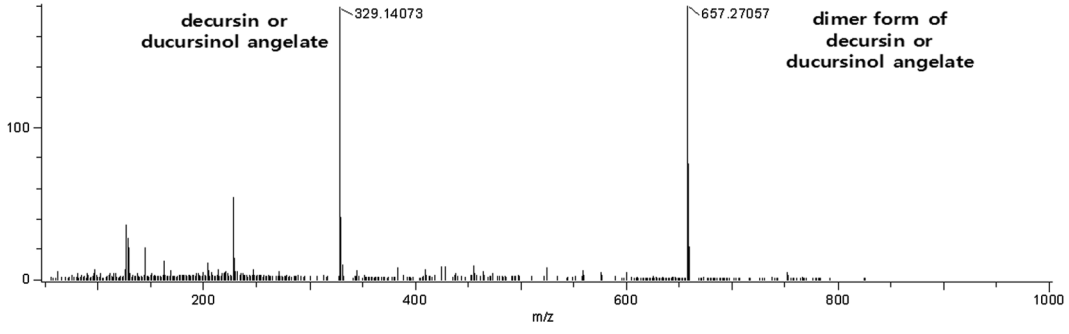

Fig. 2. Mass spectrum of the root of *Angelica gigas*.

Table 1. The observed and calculated mass numbers of phytochemicals in the root of *Angelica gigas*

Compound	[M+H]+	Theoretical mass	Observed mass	Mass difference (mmu)
Decursin or decursinol angelate	$C_{19}H_{20}O_5$	329.13890	329.14073	1.83
Ducursin or decursinol angelate dimer	$C_{38}H_{40}O_{10}$	657.26997	657.27047	0.6

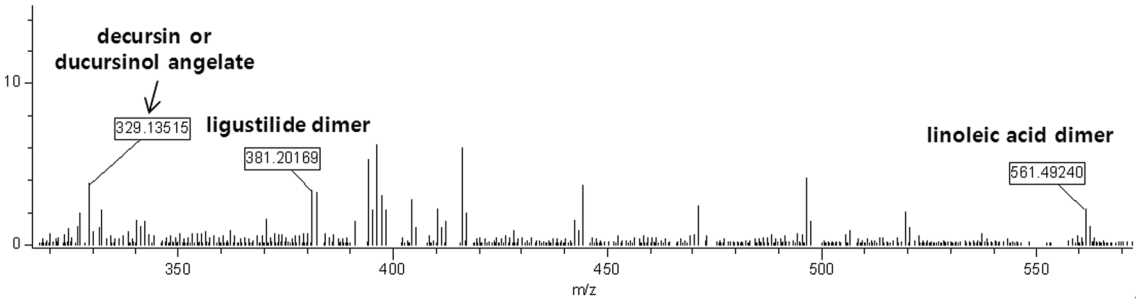

Fig. 3. Mass spectrum of the root of *Angelica acutiloba*.

Table 2. The observed and calculated mass numbers of phytochemicals in the root of *Angelica acutiloba*

Compound	[M+H]+	Theoretical mass	Observed mass	Mass difference (mmu)
Decursin or decursinol angelate	$C_{19}H_{20}O_5$	329.13890	329.14073	−3.74
Ligustilide dimer	$C_{24}H_{29}O_4$	381.20659	381.20169	−4.89
Linoleic acid dimer	$C_{36}H_{65}O_4$	561.4883	561.49420	4.11

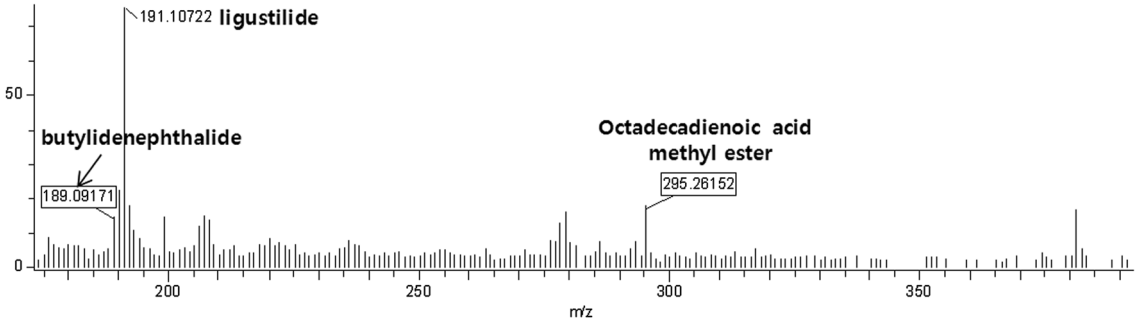

Fig. 4. Mass spectrum of the root of *Angelica sinensis*.

MS (Yang *et al.*, 2004). As shown in Fig. 3, decursin or decursinol angelate was also detected from Japanese Angelica. This result is in agreement with a previous study that utilized GC-MS analysis (Piao *et al.*, 2007). The protonated dimer of ligustilide and linoleic acid were also annotated with high accuracy in the *A. acutiloba* sample (Table 2).

The main peak at *m/z* 191.1072 in the *A. sinensis* sample was specified as protonated ligustilide, which was previously reported as the main component from *A. sinensis* (Lu *et al.*, 2005, Zhao *et al.*, 2003). The dimer peak of ligustilide was also detected (Fig. 4). The protonated ion peak at *m/z* 189.0917 and 295.2615 were determined to be butylidenephthalide and octadecadienoic acid methyl ester, respectively (Table 3) The presence of these volatile components have also been reported in a previous GC-MS study of the same species (Guan *et al.*, 2008, Lu *et al.*, 2005).

Since DART can directly ionize compounds from the sample surface, this method can be used for the rapid and efficient identification of biologically important compounds in crude herbal drugs, which is hardly possible using any other approach. Significantly different patterns of chemical fingerprints from each Angelica species could be efficiently employed to identify the exact origin within a few seconds.

Table 3. The observed and calculated mass numbers of phytochemicals in the root of *Angelica sinensis*

Compound	[M+H]+	Theoretical mass	Observed mass	Mass difference (mmu)
Butylidenephthalide	$C_{12}H_{13}O_2$	189.09155	189.09171	0.16
Ligustilide	$C_{12}H_{15}O_2$	191.107205	191.0720	0.01
Octadecadienoic acid methyl ester	$C_{19}H_{35}O_2$	295.263706	295.26152	−2.18

Conclusion

In this study, we developed a fast and efficient method for the identification of three different species of Angelica roots using DART-TOF-MS. This method successfully provided a fast discrimination of related raw materials with highly resolved chemical fingerprints in only a few seconds of analysis. Although discrimination of the three Angelica root species has been achieved using other analytical tools, DART-TOF-MS fingerprinting was much more rapid and simple than these previous methods. Considering the current trend of using chemical fingerprints as a tool for quality control of botanical drugs and herbal medicinal products, this method can be effectively utilized in this field of science.

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References

Cody, R.B., Laramee, J.A., and Durst, H.D., Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **77**, 2297-302 (2005).

Guan, Z.Y., Pu, C.H., Liu, N., Zhao, K.J., and Ding, J.C., The extraction of chemical constituents from Angelica sinensis with supercritical CO_2 fluid and the constituents analysis. *Shipin Yu Yaopin,* **10** (2008).

Hatano, R., Takano, F., Fushiya, S., Michimata, M., Tanaka, T., Kazama, I., Suzuki, M., and Matsubara, M., Water-soluble extracts from Angelica acutiloba Kitagawa enhance hematopoiesis by activating immature erythroid cells in mice with 5-fluorouracil-induced anemia. *Exp. Hematol.* **32**, 918-24 (2004).

Hou, Y.Z., Zhao, G.R., Yuan, Y.J., Zhu, G.G., and Hiltunen, R., Inhibition of rat vascular smooth muscle cell proliferation by extract of Ligusticum chuanxiong and Angelica sinensis. *J. Ethnopharmacol.* **100**, 140-4 (2005).

Kim, H.J. and Jang, Y.P., Direct analysis of curcumin in turmeric by DART-MS. *Phytochem. Anal.* **20**, 372-7 (2009).

Liu, J., Burdette, J.E., Xu, H., Gu, C., Van Breemen, R.B., Bhat, K.P., Booth, N., Constantinou, A.I., Pezzuto, J.M., Fong, H.H., Farnsworth, N.R., and Bolton, J.L., Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J. Agric. Food Chem.* **49**, 2472-9 (2001).

Lu, G.H., Chan, K., Liang, Y.Z., Leung, K., Chan, C.L., Jiang, Z.H., and Zhao, Z.Z., Development of high-performance liquid chromatographic fingerprints for distinguishing Chinese Angelica from related umbelliferae herbs. *J. Chromatogr. A* **1073**, 383-92 (2005).

Madhusudanan, K.P., Banerjee, S., Khanuja, S.P., and Chattopadhyay, S.K., Analysis of hairy root culture of Rauvolfia serpentina using direct analysis in real time mass spectrometric technique. *Biomed. Chromatogr.* **22**, 596-600 (2008).

Mcewen, C.N., Mckay, R.G., and Larsen, B.S., Analysis of solids, liquids, and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments. *Anal. Chem.* **77**, 7826-31 (2005).

Petucci, C., Diffendal, J., Kaufman, D., Mekonnen, B., Terefenko, G., and Musselman, B., Direct analysis in real time for reaction monitoring in drug discovery. *Anal. Chem.* **79**, 5064-70 (2007).

Piao, X.L., Park, J.H., Cui, J., Kim, D.H., and Yoo, H.H., Development of gas chromatographic/mass spectrometry-pattern recognition method for the quality control of Korean Angelica. *J. Pharm. Biomed. Anal.* **44**, 1163-7 (2007).

Takats, Z., Wiseman, J.M., Gologan, B., and Cooks, R.G., Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **306**, 471-3 (2004).

Yang, L., Zhong, F., Hu, S., Ji, L., and Fu, G., Comparison of volatile oil chemical constituents between Radix *Angelica sinensis* and *A. acutiloba*. *Zhongcaoyao* **35**, 1093-1095 (2004).

Yook, C.S. Coloured medicinal plant of Korea, Seoul, Academy Book Co. (1990).

Zhao, K.J., Dong, T.T., Tu, P.F., Song, Z.H., Lo, C.K., and Tsim, K.W., Molecular genetic and chemical assessment of radix Angelica (Danggui) in China. *J. Agric. Food Chem.* **51**, 2576-83 (2003).

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