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Identification of Marker Compounds in Herbal Drugs on TLC with DART-MS

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This study was conducted to provide a more versatile and specific information on Thin Layer Chromatographic (TLC) analysis of medicinal plants. TLC plates developed with the extract of herbal medicines were analyzed with direct analysis in real time (DART) ion source. Three well known herbal drugs were extracted and developed on a silica-coated TLC plate with the conditions pre-established in Korean Pharmacopoeia IX. The developed plate was placed between the DART ion source and TOF-MS analyzer to get real time mass spectra from the bands on the TLC plate directly. The marker coumarin compounds, decursin and decursinol were successfully identified from the TLC plate developed with *Angelicae gigantis* radix, along with alkaloid compounds of rutaecarpine and evodiamine from Evodiae fructus, and lignan molecules of gomisin A, N, and schisandrin from Schisandrae fructus. This hyphenation system of TLC and DART-MS could provide unique and specific information on the major constituents of crude plant drug on TLC through uncovering high resolution mass number of each band on the TLC plate directly in real time.

Key words: DART-MS, TLC, Herbal drug analysis, *Angelicae gigantis* radix, Evodiae fructus, Schisandrae fructus

INTRODUCTION

Thin layer chromatography (TLC) is widely used as a fast and simple analytic method for various organic chemicals including pharmaceuticals, natural products, biomolecules in various areas of practice. TLC is an important analytic tool especially in regulatory issues of crude drugs and closely linked with quality control of medicinal herbal drugs. TLC technique is usually included in the Pharmacopoeia of many countries (Waksmundzka-Hajnos, 2008). Although TLC has been routinely adopted for the simple identification of target compounds and visual fingerprints of herbal medicinal products for the purpose of quality control, the lack of specific and absolute information from the TLC plate limits its universal applications in phytochemical analysis. In order to overcome this shortcoming of TLC technique, various detecting instruments including diode-array detector have been tried to be hyphenated with TLC (Wilson, 1999; Spangenberg et al., 2002). Amongst hyphenation detecting techniques, mass spectrometry has been the most versatile and specific instrumentation that could provide high resolution mass number of compounds on TLC plate hence gives the identification of compounds (Wilson, 1999).

Mass spectrometry, one of the indispensable instruments in natural sciences, has been engaged to identify and quantify specified molecules on TLC plate and direct analysis of organic molecules on TLC plate *in situ* was technically possible when the totally new ion source of ambient desorption ionization was introduced (Morlock and Schwack, 2006, Van Berkel et al., 2007). DART ion source is one of open-air ionization techniques and it ionizes compounds directly in their native condition, bypassing most steps of the analytical system and transferring ions into the mass analyzer (Cody et al., 2005). Since its ionization is relatively soft as like electron spray ionization technique, DART ion source provides relatively simple mass spectra consisting mainly of protonated molecules in case of

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positive ion mode. We reported the successful application of DART-MS in the field of natural products analysis in previous article (Kim and Jang, 2009).

In this paper, we report a pilot study of quality control for crude herbal drugs using TLC with DART-MS to provide more accurate identification of marker compounds of each herbal medicine. Three well known herbal drugs, *Angelicae gigantis* radix, Evodiae fructus and Schisandrae fructus were selected for this study and their standard TLC monographs with DART-MS spectra have been elaborated.

MATERIALS AND METHODS

Chemicals and materials

The radix of *Angelica gigas* Nakai (Umbelliferae) from Korea, fruit of *Evodia rutaecarpa* Bentham (Rutaceae) from China and the fruit of *Schisandra chinensis* Baillon (Schisandraceae) from China were purchased from a domestic Korean market (Kyungdong Crude Drugs Market) in August 2007. The identities of the herbal medicines were confirmed by one of the authors (YPJ) and an expert in herbal drug discrimination. Voucher specimens of *A. gigas* (KHUP-107), *E. rutaecarpa* (KHUP-525), and *S. chinensis* (KHUP-521) were deposited in the Museum of Korean Crude Drugs located in College of Pharmacy, Kyung Hee University, Seoul, South Korea. All solvent for the extraction and development of TLC were purchased from Duksan pure chemicals Co. TLC was performed using glass-backed plates pre coated with a silica gel 60-F_{254} from Merck. For the detection of the separated spots on TLC plate, dual UV lamp from Camag carrying 254 and 366 nm was used.

Analytical conditions of DART-TOF-MS

AccuTOF-TLC single-reflectron time-of-flight mass spectrometer (JEOL) equipped with a direct analysis in real time (DART) ion source (IonSense) was used for the analysis of TLC plates and Mass center version 1.3.7 software was used to operate and process MS spectrometer and MS spectra. This model is installed with TLC carrier for more efficient analysis of TLC plate. The atmospheric pressure interface potentials were set to the following values: orifice 1 = 10 V, ring lens and orifice 2 = 10 V. The rf ion guide potential and detector voltage were set to 1000 V and 2400 V. DART electrode potentials were set to needle (glow discharge) electrode = 3000 V, electrode 1 = 100 V, electrode 2 (grid) = 100 V. Gas temperature was set to 200°C, and helium gas flow rate was 3 liters per minute. As an external reference and tuning standard, polyethylene glycol 600 (PEG 600) was used for exact mass measurements.

Extraction and development conditions for identification test

Three herbal medicines were extracted with the methods listed in Korean Pharmacopoeia IX, respectively (Korean Pharmacopoeia IX, 2008). Detailed conditions of TLC development and detection for each herbal medicine were described in Table I. The developing solvent system of *Evodia rutaecarpa* fruit was prepared from the method in Korean Pharmacopoeia IX. The developing conditions of *Angelicae gigantis* radix and Schisandrae fructus were slightly modified from those in Korean Pharmacopoeia IX for better resolution.

DART analysis of TLC plate

The developed plate was air dried and examined under UV light to localize the band corresponding to each marker standard compound. For DART analysis, the width of TLC plate was cut to around 0.5 cm. The bands of marker compounds in each herbal medicine were directly placed under the excited helium gas stream of DART ion source. When the band of marker compound was introduced into the DART gas stream, the protonated molecule of each compound was appeared on the mass spectrum in real time.

RESULTS AND DISCUSSION

Since DART ion source can ionize various organic molecules from samples directly without any extraction and sample preparation steps, the combination of

Table I. TLC conditions, detection methods, and marker compounds for each herbal medicine.

Herbal medicine	Development condition	Detection	Marker compounds
Angelicae gigantis radix	hexane : ethyl acetate : methanol = 3 : 2 : 1	UV 365 nm	decursin, decursinol
Evodiae fructus	dichloromethane : methanol : formic acid = 40 : 1.5 : 2	ethanolic H_2SO_4 UV 365 nm	evodiamine, rutaecarpine
Schisandrae fructus	toluene : ethyl acetate : formic acid = 7 : 3 : 0.5	ethanolic H_2SO_4 UV 365 nm, 254 nm	gomisin A, N, schisandrin

DART ion source with TLC would be an efficient system for the identification of marker compounds in crude herbal drugs for their identification and quality control. We proved the simplicity and power of this herbal drug analysis system by representing the simple TLC separation and specific analysis of the marker compounds in an extract of three medicinal plants on TLC with DART-MS analysis.

The chemical structures of seven marker compounds were shown in Fig. 1. The spots of pyranocoumarin, decursin and decursinol from the extract of *A. gigas* were successfully separated on TLC and showed greenish blue fluorescence upon UV 356 nm illumination (Fig. 2). In Korean Pharmacopoeia IX, decursin and decursinol are listed as marker compounds. The protonated molecular ions of these marker coumarin com-

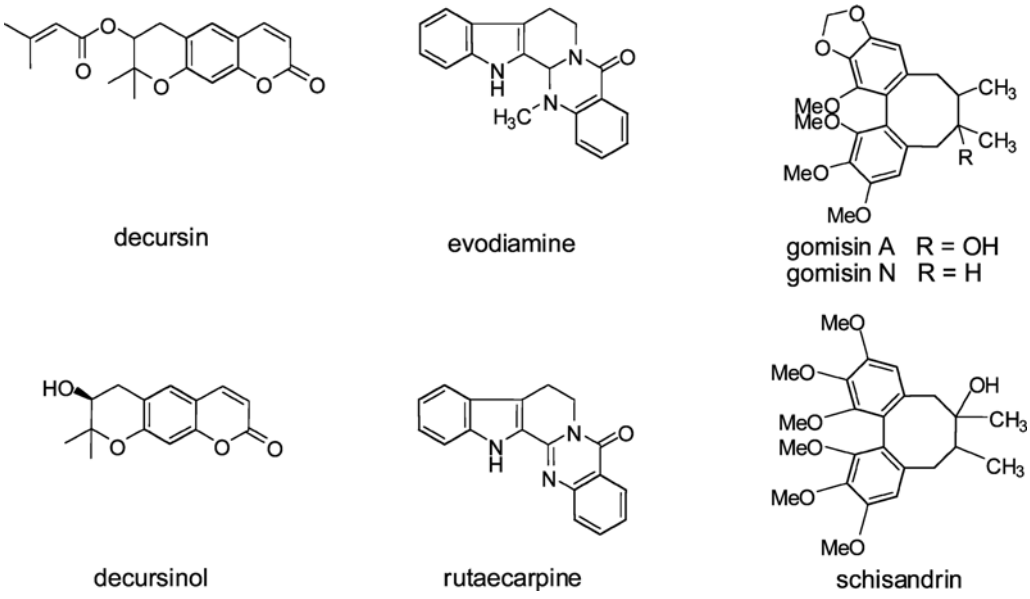

Fig. 1. Chemical structures of marker compounds listed in three medicinal herbal drugs

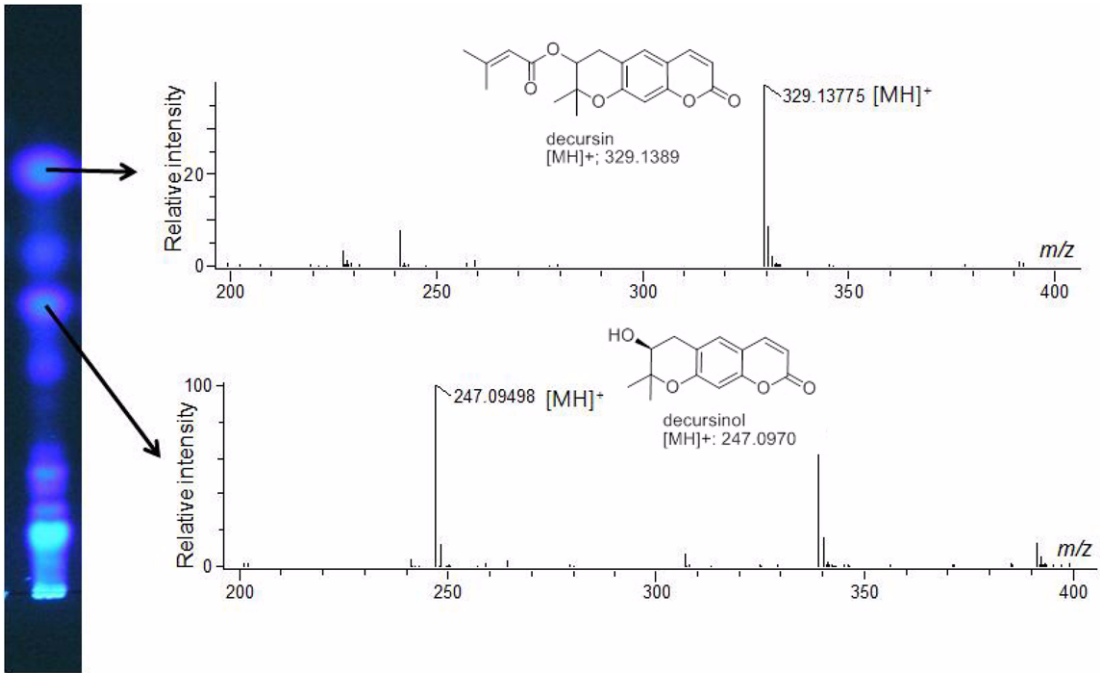

Fig. 2. TLC chromatogram from the extract of *Angelicae gigantis* radix and DART-MS spectra of decursin and decursinol. TLC plate was visualized under UV light of 365 nm.

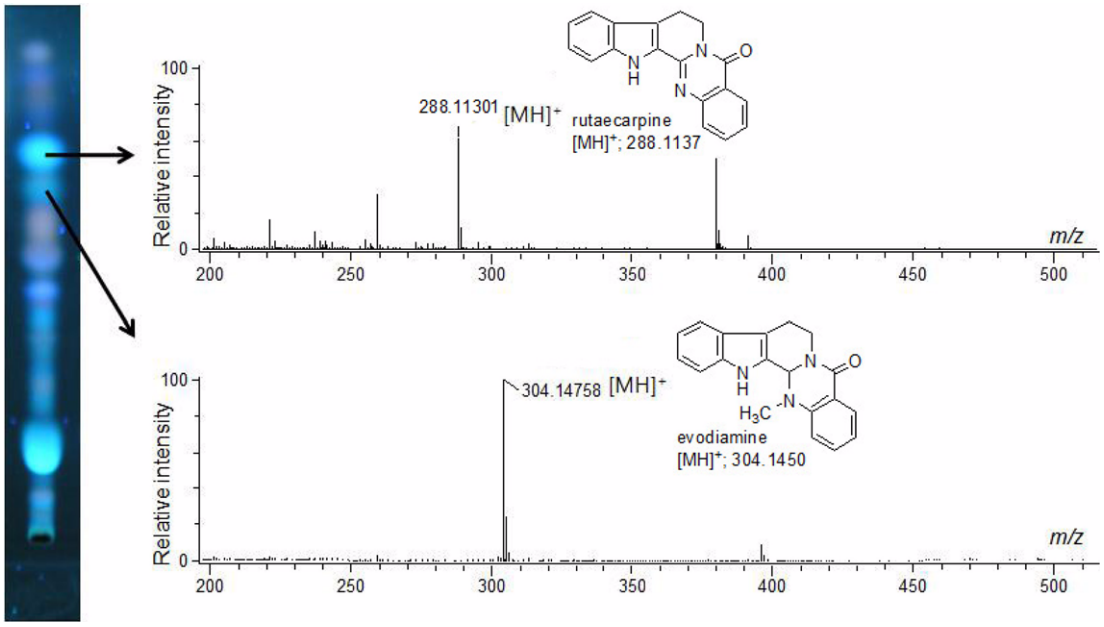

Fig. 3. TLC chromatogram of the extract of Evodiae fructus and DART-MS spectra of rutaecarpine and evodiamine. TLC plate was visualized under UV light of 365 nm after heating with ethanolic H_2SO_4 spray reagent.

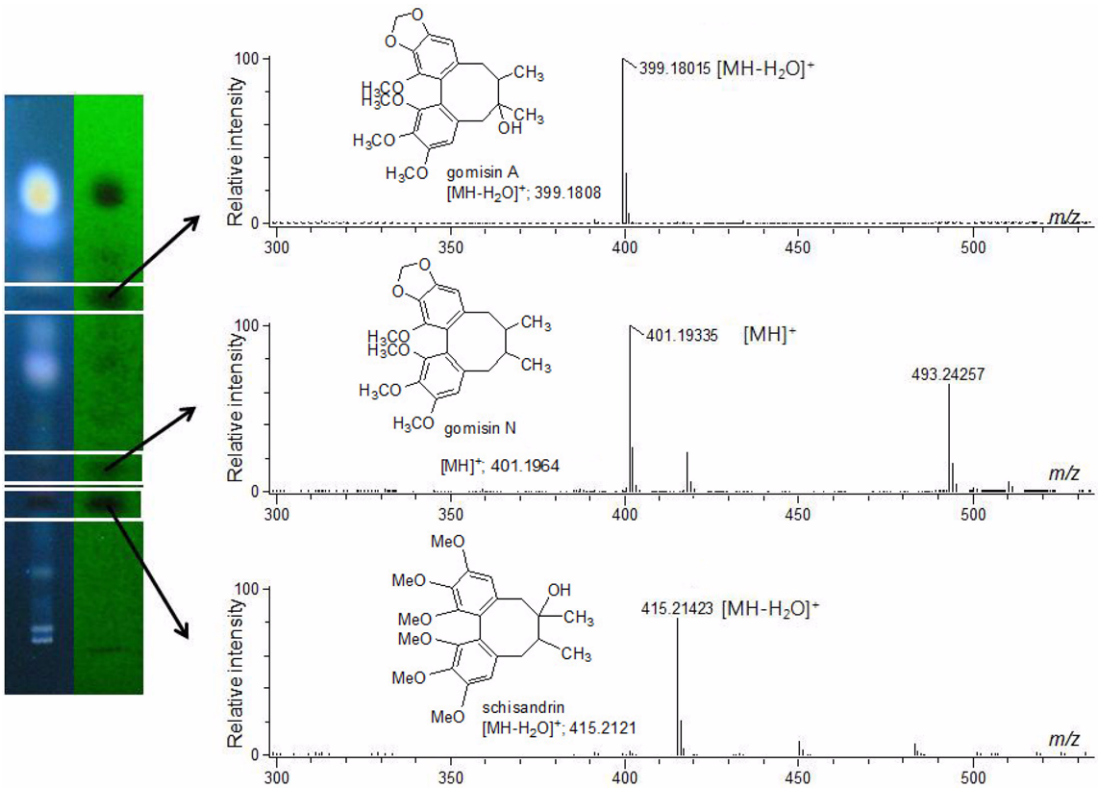

Fig. 4. TLC chromatogram of the extract of Schisandrae fructus and DART-MS spectra of gomisin A, N, and schisandrin. TLC plates were visualized under UV light of 365 nm after heating with ethanolic H_2SO_4 spray reagent (left) and under UV light of 254 nm (right).

pounds were successfully detected from the spots by a few seconds of analysis with DART-MS. In the lower panel of DART-MS spectra, some other compound having molecular weight of around *m/z* 340 was also seen in the spectra along with the protonated molecule of decursinol. It showed that the band of decur-

sinol is not composed of decursinol only and this feature of the system could be another benefit for the analysis of crude herbal drugs.

From the DART-MS spectra of the TLC plate developed with the extract of Evodiae fructus, corresponding molecular peaks of evodiamine, rutaecarpine were observed from the designated spots, respectively (Fig. 3). In upper panel of DART-MS spectra, other compounds were also found with rutaecarpine.

Schisandra lignans in the extract of Schisandrae fructus were visualized under UV light 254 nm as dark-brown spots (Fig. 4, the right TLC plate). The protonated molecules of gomisin A and schisandrin appeared at m/z 399 and 415, respectively. These values were both m/z 18 less than their original protonated molecules 417 and 433. This phenomenon was commonly observed in mass spectroscopic analysis of samples containing secondary or tertiary alcohol (Deng et al., 2008). During the ionization process of these molecules in gaseous state, dehydration reaction often occurs to give dehydrated molecular ion peaks. In DART-MS analysis, protonated molecule of the compound with secondary or tertiary alcohol group tends to be dehydrated during ionization reaction, too. This was easily shown with DART-MS analysis of cholesterol as the dehydrated molecular ion peak of m/z 369 was the main peak instead of original protonated molecular ion peak of cholesterol, m/z 387 (data not shown). Contrast to these compounds, gomisin N which has no free hydroxyl group in the structure was detected as protonated form $[M+H]^+$.

Besides the identification of known marker molecules from crude herbal drugs, the DART-MS application on TLC plate can be utilized in producing chemical fingerprint for the quality control of herbal medicines. DART-MS analysis of TLC would provide a simple and prompt but highly specific and valuable information about various components in crude herbal drugs.

CONCLUSION

This study described a new analytic system of TLC hyphenated with DART-MS for the rapid identification of phytochemicals in herbal drugs. This system successfully provided real time information of high-resolution mass number of compounds on TLC plate. Since TLC method is still in general use for analysis of various natural products and for its simplicity and convenience, the hyphenation with DART-MS as a detecting tool will potentially expand its usages in various analyses. Considering the current trends of the establishment of chemical fingerprint as a tool for the quality control of botanical drugs and herbal medicinal products, DART-MS analysis of crude herbal drug on the TLC will be a good analytical system to make such a fingerprint for the efficient and reliable quality control of medicinal herbal products and crude herbal drugs.

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