DOI: 10.15806/j.issn.2311-8571.2016.0001 World J Tradit Chin Med 2016; 2(1): 2–9

DART-MS: A New Research Tool for Herbal Medicine Analysis

Yao Shen, Wan-Ying Wu and De-An Guo*

Shanghai Research Center for Modernization of Traditional Chinese Medicine, National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Haike Road 501, Shanghai 201203, China
**Correspondence*: De-an Guo, Prof and Director, Institute of Materia Medica, Chinese Chinese Academy of Sciences, Shanghai 201203, China.
E-mail: daguo@simm.ac.cn

ABSTRACT

Direct analysis in real time (DART) possesses the merits of analyzing sample in its native status with minimal or even no sample pretreatment. In this review, we summarized the recent applications of DART in the field of herbal medicine analysis such as compound detection, species identification, metabolites profiling and initial quantification. DART with the characters of hyper-rapid, easy-hyphenated offers a new research tool for herbal medicines to complete the experimental process in a very simple but still reliable way. It is anticipated that more wide and deep applications of DART in herbal medicine analysis, as rapid quantification, high-throughput active compounds screening, rapid species identification, and fast illegal additives screening will be promising and foreseeable in the near future.

Key words: Ambient mass spectrometry, Direct analysis in real time (DART), Herbal medicines, Species identification
Received 4 January 2016; Accept 28 February 2016

INTRODUCTION

Mass spectrometry (MS) is one of the most powerful techniques for herbal medicine analysis due to its high resolution and/or fragment ions information. Traditional mass spectrometry is powerful for uncovering information of medicinal herbs. However, problems like matrix effect, ionization efficiency, tedious sample pretreatments, still limit its wide applications.

Recently appeared new ionization technique which is called ambient mass spectrometry may bring mass spectrometry applications to a new milestone. Ambient mass spectrometry is introduced as analysis of samples with minimal or even no sample pretreatment and ionization of sample in its native status with atmospheric surroundings. Since the first ambient ionization source Desorption Electrospray Ionization (DESI) has been introduced in 2004 by Graham Cooks [1], around 50 sorts of source version have been reported [2]. One of the most commercialized versions is Direct Analysis in Real Time (DART) which was introduced in 2005 [3]. DART and its ionization mechanism are shown in Figure 1. DART ionization source works at atmosphere, a heated gas flow (normally helium or nitrogen) goes through the main chamber where produce plasma of ions, electrons, metastable atoms and molecules. Most of the charged particles are screened from the neutral gas molecules by the next grids to go to the orifice. Nitrogen gas turns to excited gas N_2*, then transfer energy to the analyte S that has lower ionization potential than the energy of N_2*, which produces a radical molecular cation $S^{+\cdot}$ and an electron (e^-). This Penning ionization mechanism is the main reaction mechanism when nitrogen or neon is used as the gas in DART positive mode. Helium is more recommended gas for DART due to its high ionization energy at 19.8 eV, which can efficiently react with water in the atmosphere. The positive-ion formation mechanism of it includes ionization of water clusters $[(H_3O)_nH]^+$ followed by proton transfer from water cluster to thermal desorbed samples $[M+H]^+$. Negative-ion formation has no difference between nitrogen, neon or helium. Electrons (e^-) formed by Penning ionization or surface Penning ionization are captured by oxygen in the atmosphere to produce O_2^- which finally reacts with the analyte S to form anions $[M-H]^-$.[3]

Since DART source has been introduced, it was widely applied in biochemistry, forensics, natural products, explosives, pharmaceuticals etc. [5–11] DART-MS showed great advantages for analysis of sample with complex matrix, e.g., blood, plants, animal tissue, enzyme-substrate complex, because of no requirement for any pretreatment and separation. This feature may give a chance to move herbal medicine researches from the traditionally tedious and time-consuming manner to a fast and direct way. In this review, we will give a summary on the recent herbal medicine analysis by using DART-MS, and present a perspective of DART-MS for the future applications in herbal medicines.

QUALITY CONTROL OF HERBAL MEDICINES BY QUALITATIVE DART-MS

1. Detection of compounds in herbal medicines

Alkaloids distribution in root, stem, flower, leaf and pod of *Prosopis juliflora* was investigated by using DART-HRMS without any sample pretreatment by Shachi et al. [12] Different groups of alkaloids e.g., piperidine alkaloids, prosopine and prosopinine alkaloids, are identified and their relative abundance were calculated for presenting their distribution in

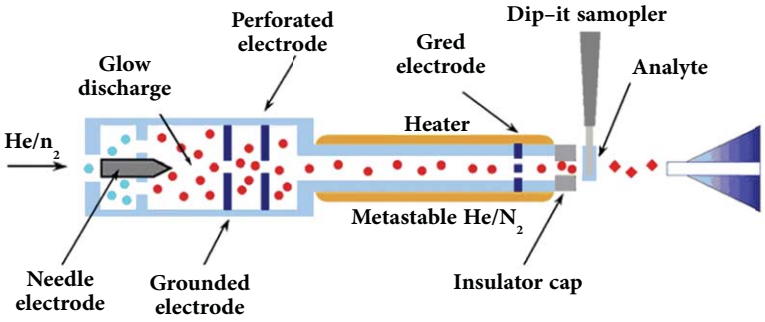

Figure 1. Diagram of Direct Analysis in Real Time (DART) source. Reprinted with permission from [4]. Copyright (2011) Elsevier

different parts of *Prosopis juliflora*. Alkaloids, flavonoids and ginsenosides were quickly identified by DART-TOF/MS by Liu and coworkers.[13] All samples were only extracted by methanol-water (1:1) in ultrasonic bath for 2 min. Corydaline, tetrahydropalmatine from *Corydalis yanhusuo* extract; peimine, peiminine from *Fritillaria pallidiflora* extract; arecaidine, arecoline from *Areca catechu* extract; rhynchophylline from *Uncaria rhynchophylla* extract; and baicaleine, wogonin from *Scutellaria baicalensis* extract were rapidly detected by DART-TOF/MS. Detected ions were all present as $[M+H]^+$, which makes the spectrum simple. Ginsenosides with high volatility e.g., pseudoginsenoside F11, coumpound K, protopanaxatriol, and protopanaxadiol can be directly detected by DART-MS. However, ginsenosides with low volatility need a derivatization step before putting in front of the orifice. Derivatized ginsenoside ions (e.g., methylated ginsenoside Rc) were obtained by DART-MS in high abundance. Song and coworkers[14] detected 15 alkaloids in Aconiti Kusnezoffii Radix and Aconiti Radix by DART-MS. Even thermal sensitive diester diterpenoid aconitines may decompose during ionization, their fragment ions, like $[M+H-H_2O]^+$ and $[M+H-CH_3COOH]^+$ could also be assistant for identification. Saccharide is an important chemical group in herbs because of their anticancer, cellular recognition, antiviral properties, etc. Liu and coworkers[15] investigated different types of saccharides such as monosaccharides, disaccharides and trisaccharides by DART-MS under different temperatures in positive mode. Sugar clusters and ammonia adducts ($[M+NH4]^+$) were easily observed for monosaccharides, disaccharides standards by increasing gas temperature. Ammonia adducts $[M+NH_4]^+$, however, are hardly formed by DART-MS for trisaccharides and real sample ginseng oligosaccharide extract without derivatization. Rahman et al. [16] detected 15 compounds in turmeric rhizomes by DART-MS in both positive and negative modes, and also found that curcumin and demethonycurcumin are mainly located in the pith rather than other parts of turmeric rhizomes. Besides, a simplified DART source version combined with high resolution mass, ID-CUBE-HRMS was used to identify phenolic compounds in raw leaves of *Bergenia crassifolia* L. in negative mode [17]. More information about the detected compounds in herbal medicines by DART-MS was summarized in Table 1.

Applications of DART for detection and identification of compounds in herbal medicines were characterized to be fast, simple and reliable, which shortened the analysis time in a great degree. DART identification is direct (*in situ*) and time-saving, however, it normally needs the presence of high resolution mass or tandem mass as the detector for discriminating from their interferents (see Table 1). In addition, analytes with the same molecular weight are not possible to be distinguished. Examples in this section proves that DART has the potential to quickly and qualitatively detect compounds in herbal medicines. It is noteworthy that protonated ions or ammonium adducts are the main format of ions in DART positive mode, and deprotonated ions are the main format of ions in DART negative mode, which give a relatively simpler mass spectra for sorting out chemical information afterwards. More DART-related techniques and methodologies will be introduced in the following sections to show the potential of DART in quality control of herbal medicines.

2. Identification of herbal medicine species

One of the outstanding features for herbal medicines, also for natural medicines, is their biodiversity, which gave unlimited possibilities to produce secondary metabolites with the potential for healing diseases. Herbal medicines not only take the advantages from its great biodiversity, but also are hampered by it. This is due to more biodiversity of herbs give more possibilities to produce active compounds for medicinal use. However, on contrast, more biodiversity sometimes made quality control of herbal medicines more difficult and complicated. Conventional methods for identification of herb species includes dissecting microscope operated by professional botanists, spectroscopic techniques (NMR, Raman, IR, etc.) and mass spectrometric related techniques (LC-MS, CE-MS, etc.) for gathering chemical information in addition with time-consuming sample pretreatments, and DNA sequence analysis that is only suitable for which gene sequence information is known [22]. These techniques are useful, but not that efficient. Recently, DART with the advantage of

Table 1. Compounds detected by DART-MS in herbal medicine analysis

Herbal medicines	Devices	Mode	Detected compounds	Reference
Piper betle	DART-TOF-MS	Positive	Phenols and their acetates: chavicol, allylpyrocatechol, chavibetol, chavicol acetate, allylpyrocatechol acetate, chavibetol acetate, allylpyrocatechol diacetate	[18]
Aconitum carmichaelii and *Aconitum kusnezoffii*	DART-LTQ /Q-TOF-MS	Positive	Alkaloids: songorine, isotalatizidine, talatizamine, neoline, fuziline, mesaconine, benzoylhypaconine, benzoylmesaconine, benzoylaconine, hypaconitine, deoxyaconitine, mesaconitine, aconitine, 10-OH-mesaconitine, 10-OH-aceonitine	[14]
Panax ginseng	DART-TOF-MS	Positive	Saccharides: glucose, mannose, galactose, rhamnose, arabinose, xylose, sucrose, trehalose, maltose, cellobiose, lactose, raffinose, ginseng tetrasaccharide,	[15]
Corydalis yanhusuo, Fritillaria pallidiflora, Areca catechu, Uncaria rhynchophylla, and *Scutellaria baicalensis*	DART-TOF-MS	Positive	Alkaloids, flavonoids and ginsenosides: corydaline, tetrahydropalmatine, peimine, peiminine; arecaidine, arecoline, rhynchophylline, baicaleine, wogonin, pseudoginsenoside F11, coumpound K, protopanaxatriol, protopanaxadiol, methylated ginsenoside Rc	[13]
Curcuma longa	DART-LTQ	Positive & negative	Curcumin, alphaturmerone, dihydro-ar-turmetone, (E)-alpha-atlantone, 1,3-dihydroisobenzofuran, caffeic acid, methyl 8,11-octadecadienoate, ar-turmerone, (E)-3-methyl-6-p-tolylhepta-1,4-dien-3-ol, 6-(3-hydroxy-4-methylcyclohexa-2,4-dienyl)-2-methylhept-2-en-4-one, 7-(cyclohexa-1,3-dienyl)-5-hydroxy-2,6-dimethylhept-2-en-4-one, n-hexadecanoic acid, linoleic acid	[16]
Bergenia crassifolia	ID-CUBE-Orbitrap	Negative	Phenolic compounents: hydroquinone, gallic acid, arbutin, ellagic acid, bergenin, fumaric acid, pyrogallol, malic acid, quinic acid, trihydroxycoumarin, acetylsalicylic acid, furancarboxylic acid, bergapten, norathyriol, norbergenin	[17]
Prosopis juliflora	DART-TOF-MS	Positive	Alkaloids: Juliprosopin, juliprosine, prosoflorine, juliprosinine, 3-Oxo-juliprosine, 3``-Oxo-juliprosopine, prosopine, prosopinine, julifloridine, projuline, prosafrinine, N-methyl-julifloridine	[12]
Cinnamomum tamala	DART-TOF-MS	Positive	polyphenols and terpenoids: cinnamaldehyde, p-cymene, camphene, limonene, phellandrene, cinnamic acid, carvone, camphor, linalool, eugenol, cinnamyl acetate, methyl eugenol, eugenol acetate, β-caryophyllene oxide	[19]
Berberis petiolaris	DART-TOF-MS	Positive	Alkaloids: berberrubine, demethyleneberberine, reticuline, berberine, jatrorrhizine, tetrahydroberberine, magnoflorine, 8-oxo-berberine, palmatine, N-methyltetrahydroberberine, tetrahydropalmatine	[20]
Re Du Ning Injections	DART-Q-TOF-MS	Positive & negative	Iridoid glycosides and caffeoylquinic acids: geniposide, secoxyloganin, ranolazine, 4-amino-6-chloro-1,3-benzenedisulfonamide, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid,	[21]

simple and quick data collection, minimal or even no sample pretreatment combined with chemometrics showed their potential in identification of herbal medicine species.

Herbs belonging to the same genus but different species normally possess similar morphology and chemical composition. This similarity normally leads to misapplication or adulteration of medicinal herbs. Discrimination of morphologically similar herbs by using marker compounds or fingerprints is frequently employed by mass spectrometric related techniques. The ideal situation is that the feature compound only presents in one of the two similar species. Teris and his coworkers[23] presented a good example in this case. Two star anise species, Chinese star anise fruits (*Illicium verum*) together with Japanese star anise (*Illicium anisatum*) are used as the source of shikimic acid which can be synthetically converted to Tamiflu. Chinese star anise is also used as cuisine in Asia and tea flavor in Europe. However, morphologically similar Japanese star anise containing a neurotoxin, anisatin, at a toxic level, makes the distinction of them indispensable. DART-HRMS gave high response of anisatin in Japanese star anise but almost no signal of it in Chinese star anise. Compared to the traditional 12-step pretreatment and LC-MS/MS analysis, identifications by DART-HRMS is much faster and the result is robust. Fukuda and his coworkers [24] also distinguished *Glycyrrhiza inflate* from *G. glabra* and *G. uralensis* by detecting the specific compound of *G. inflate*, licochalcone A in positive mode of DART-MS.

In most of the cases, however, feature compounds rarely show "yes" or "no" difference between confusing species. They may be present in each species but with different content. In this case, profile or response abundance plays as the crucial factor for discrimination of morphologically similar species. This profile is normally named as "fingerprinting". Conventional technique consists of solvent extraction, liquid chromatographic separation and detection, which

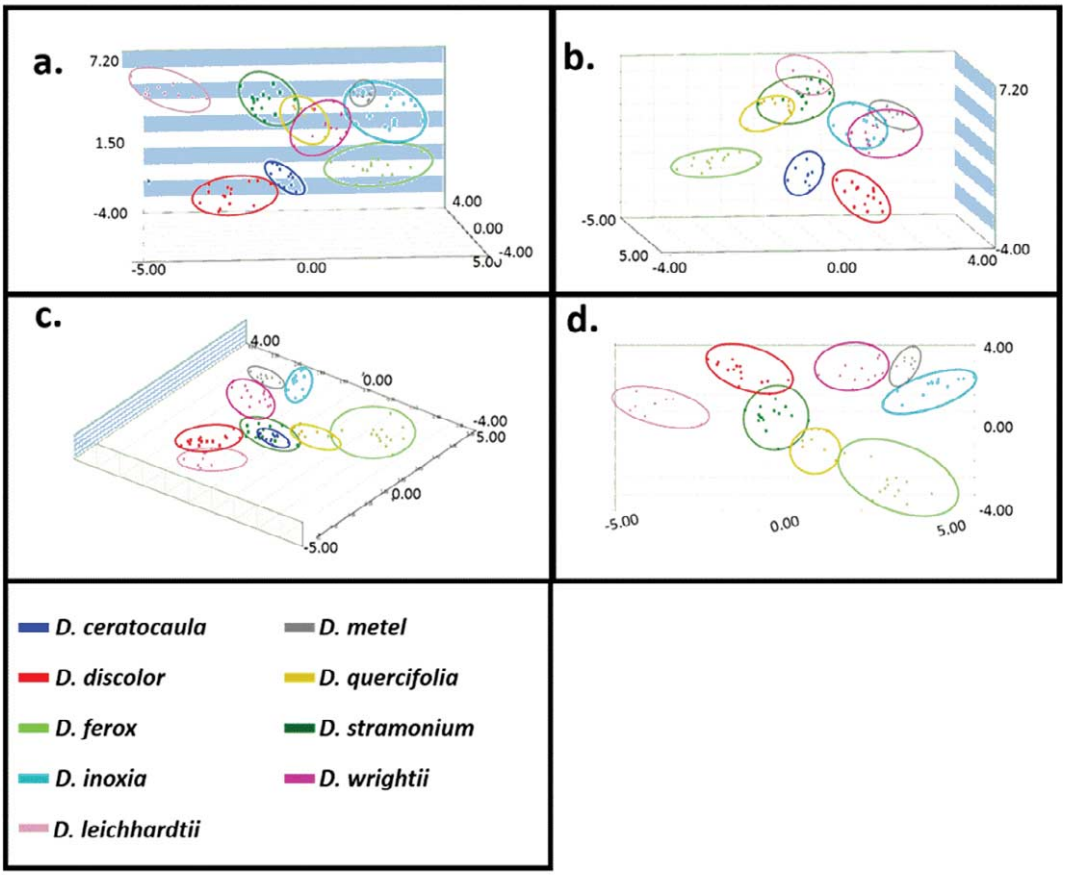

Figure 2. Linear discriminant analysis (LDA) plot of mass spectral data derived from DART-HR-TOF-MS for analysis of *Datura* spp. Seeds. Reprinted with permission from [28]. Copyright (2015) American Chemical Society.

normally takes hours to get a "fingerprinting". Recently, fingerprinting collected by DART-MS combined with chemometrics also enabled species discrimination. Jang and coworker[25] obtained the fingerprinting of *Litsea cubeba* by DART-TOF-MS, with the auxiliary of principal component analysis (PCA), samples collected from the market can be confirmed as *Litsea cubeba* or its similar species *Piper cubeba*. They also classified four *Umbelliferae* species by DART-TOF-MS and multivariate analysis. Fingerprinting of *Angelica tenuissima*, *A. gigas*, *A. dahurica* and *Cnidium officinale* were collected by putting the packed powder directly to the orifice without any sample pretreatment [26]. Not only the mature herbs, but also the herb seeds [27] can be identified by DART-HRMS. Musah et al. [28] demonstrated the utility of DART-HRMS fingerprinting in *Datura* seeds classification. Linear discriminant analysis (LDA) scatter plot of mass spectral data derived from DART-HRMS successfully resolved four different *Datura* species, *D. ceratocaula*, *D. discolor*, *D. ferox*, and *D. leichhardtii*, from one another (see Figure 2a and 2b). This is due to the relative proportion of biomarkers to be different from species to species. Eight principal components contribute to 98.02% of the variance. Figure 2d showed the image of LDA plot without *D. ceratocaula* cluster. Other eight species were well resolved from each other. Compared to the conventional metabolomics, genomic technique or 18 h alkaloids analysis method for *Datura* seeds identification, DART-HRMS fingerprinting is faster, high-throughput and no need to be limited to known biomarkers.

The above markers or fingerprinting data derived from DART-MS analysis combined with multivariate data analysis demonstrated good applications of DART in herbal medicine species identification. Some of them compared the raw herb fingerprinting to its extract obtained by DART-MS. These two fingerprints share consistence but with exceptions. In the case of *Datura* seeds, Musah et al. [28] found that solvent extraction may exist different uptake capacity for various compounds in the same group. A suitable solvent or mixed solvent with maximum uptake capacity for the analytes of interest need time-consuming optimization, which means direct analysis of raw solid samples to be superior. Superiority is also shown as raw solid sample provides the same or even better result than its extract without requirement of optimization. More applications for herb species identification were also expanded to a wider range of herbs [29–30].

3. Traceability and monitoring of herbal medicines

The same herbal species but growing in different geographic conditions or processed in different ways also make differences between them. For example, the same species of *Angelica gigas* was cultivated both in China and Korea. Jang et al. [31] established a fast DART-TOF-MS method to distinguish them and avoid adulteration. The 120 samples collected from China

and Korea were analyzed by DART-TOF-MS without sample pretreatment. Variable important compounds for orthogonal partial least squares discriminant analysis (OPLS-DA) study were selected as markers for future distinction. Quick origin prediction test for blind-external *Angelica gigas* samples was accomplished based on the mass spectrometry data derived from DART-TOF-MS. Song and coworkers [32] rapidly differentiate grape seeds (*Vitis vinifera*) from different origins by using chemical fingerprinting information obtained by DART-TOF-MS in negative mode. Twenty compounds in grape seeds were identified. Two groups of clusters were separated due to chemometric calculation. Higher content of gallic acid, myricetin, and ampelopsin in Cluster I than in Cluster II, and in reverse, lower content of caftaric acid and apigenin in Cluster I than in Cluster II implies possibly different functionality of grape seeds in different origins. Thereby, this may give information for the efficient utilization of grape seeds. Fukuda et al. [33] distinguished Chinese and Japanese *Sophora flavescens* by chemical fingerprints derived from DART-MS and statistical volcano plot calculation.

Liu et al. [34] discriminated the unprocessed or improperly processed Aconiti Radix from the SOP processed ones by DART-MS and chemometrics. The relative intensities of DDAs (diester diterpenoid aconitines) obtained by DART-MS are the most relevant factor to distinguish qualified and unqualified Aconiti Radix. Qu et al. [35] performed the fingerprinting analysis of *Danshen* injection by DART-MS. With the help of multivariate data analysis, *Danshen* injections produced by different manufacturers were separated into groups. Ions contribute to the PCA result were selected as potential quality control markers. Conventional LC/UV or LC/ELSD confirmed the suitability of these selected markers.

Qu et al. [36–37] reported two methods for monitoring the variations during the herbal drug manufacturing process, one applied to monitor batch-to-batch repeatability for alkaline precipitation, the other applied to monitor multivariate batch percolation process. Both of them demonstrated that DART-MS is also an efficient tool for monitoring the manufacturing process. Quick and reliable DART-MS technique is applied not only for monitoring the major peaks to control the quality of herbal medicines during manufacturing, but also for supervising the illegal adulteration of herbal medicines or dietary supplements. Zhou et al. [38] established a DART-Q-TOF method for rapidly screening seven anti-diabetic drugs, glibenclamide, gliclazide, glipizide, gliquidone, metformin, nateglinide, and rosiglitazone, illegally added in the herbal supplements.

DART-MS dramatically reduces the time for collecting mass spectrometry data from hours to seconds without losing qualitative information. Data derived from DART-MS, with relatively low repeatability and high RSD value, however, failed in semi-/quantification cases in the beginning. The increasing robustness of DART in either commercial or home-made sampling way, for example, automatic mesh sampling, automatic dip-it sampling, vacuum tweezer sampling [28], tailor-modified confined interface sampling (for volatile organic compounds) [39], contributed to the increasing number of DART-MS applications for herbal medicine studies not only in discriminations but also in determinations.

QUALITY CONTROL OF HERBAL MEDICINES BY SEMI-QUANTITATIVE AND QUANTITATIVE DART-MS

Kim and coworkers [40] reported a method for rapid detecting curcumin in *Curcuma longa* and semi-quantitative analysis of curcumin in herbal supplements by using dip-it sampling and DART-MS technique. Compared to the parallel HPLC-UV method, inter- and intra-day RSD of DART-MS method are about 30 times higher than the conventional method, and R^2 is 0.9823 for calibration curve. In the case of distinguishing toxic Japanese star anise from non-toxic Chinese star anise, Teris and coworker [23] also demonstrated a semi-quantification analysis of anisatin in tea bag. The R^2 in both positive and negative modes are higher than 0.995. Besides semi-quantification, Xu et al. [41] reported a method for quick quantification of 1-deoxynojirimycin in Mulberry (*Morus alba* L.) leaves by DART-MS. External standard curve with the correlation coefficients (R^2) of 0.996 was used for quantification, MS/MS was used for confirmation. Validation parameters for DART-MS method was compared with that for conventional HPLC-FLD method. Quantification of 1-deoxynojirimycin by both DART-MS and HPLC-FLD methods in five mulberry leave samples were carried out. The result showed that quantitative result is comparable with each other. DART-MS method with slightly higher RSD of repeatability (7.05%), inter- and intra-day variation (7.29% and 11.14%), slightly lower correlation coefficient (R^2=0.996) than HPLC-FLD method, and with the reasonable recovery (87%-96%), is satisfactory for quantification. Gao et al. [42] determinated 5-hydroxymethylfurfural in a traditional Chinese herbal formulation by DART-QTOF-MS. Results obtained by DART-QTOF-MS is comparable with that by UPLC-QQQ-MS. Good correlation coefficients 0.996 and good relative standard deviations (RSD) for intra-day and inter-day precisions (1.2% and 8.3% respectively) were obtained with the help of internal standard quantification.

DART-MS coupled with HPTLC (thin-layer chromatography) is an interesting simple combination for phytochemists. TLC is a routine technique for pharmaceutical analysis, however, its hyphenation difficulty limited its applications, especially for quantification applications. DART with the advantages of surface analysis solved the hyphenation problem for TLC. In reverse, TLC makes up the disadvantage of DART with no separation. Combined with TLC, DART-MS may possess more possibilities to carry out quantification. Kim et al.[43] demonstrated a quantitative analysis of schisandrin, gomisin A, and gomisin N in Schisandrae Fructus by TLC-DART-TOF-MS. TLC plate was settled on the carrier between DART and orifice of MS. TLC plate spotted with a series of stock solution were analyzed by DART-TOF-MS. Experiments were carried out by TLC-UV and HPLC-UV at the same time. Compared to TLC-UV, TLC-DART-MS possessed eight times lower LOD and LOQ, and slightly higher coefficient (R^2). But

the TLC-DART-MS result is still not completely comparable with HPLC-UV result. Few quantitative applications above show comparable results to the conventional techniques.

Though we have not found an example by using DART-MS and internal standard quantification in herbal medicine studies, there are a few publications showing DART-MS to be successfully applied in quantification of toxins in food analysis by using isotope-labelled internal standard. Good validation parameters could be obtained by adding isotope-labelled internal standard for DART-MS quantifications. Better correlation coefficient was obtained with the correction of internal standard, even using matrix standard [8–9, 44].

DART-MS, coupled with other techniques (e.g., LC[45], TLC[46]), or used as auxiliary chemical information analyzer for herbal medicine bio-assays (e.g., *Gentiana scabra*) [47], showed its wide utilization in various herbal medicine researches.

DISCUSSION AND FUTURE PERSPECTIVE

The merits of ambient mass spectrometry as simple, time-saving, minimal or even no sample pretreatment attracted great attentions from researchers who worked on traditional techniques. As above-introduced cases, DART, as the most widely used ambient ionization source due to its extraordinary simplicity for operators, has already been used for compounds detection, species identification, metabolite profiling and initial quantifications for herbal medicine analysis. Herbal sample in its native status, e.g., intact raw herbs, grinded powder, extracts, liquid tissue, etc. can be directly analyzed by DART. Correspondingly, tweezer, automatic moving platform and dip-it were used for sampling. However, powder sample is not that recommended from our point of view since solid powder may be sucked into MS capillary tube to cause contamination or problematic outcomes. DART rapid fingerprinting combined with chemometrics accelerates traditional chromatogram-based fingerprinting methodology without losing characteristic information. In addition, protonated or deprotonated ions, ammonium adducts or potassium adducts normally present as the base peak in spectra, which simplified the mass spectra in a large degree. Quantification by using of MS is normally hampered by matrix effect, which made matrix effect is an important factor for MS. Even though, there is no evidence to show the comparison of matrix effect between conventional MS and ambient MS. However, Jacob et al. [48] compared a series of plasma based ambient ionization sources, the result shows that DART processes lower matrix effects than others. This implies that DART has slightly higher selectivity than other ambient ionization sources, but no clear explanation for it at this moment.

Characteristic mass spectra information of herbs obtained by DART-MS enabled the compounds detection, species identification and quick quantification. However, many polar compounds such as glycosides, trisaccharides and some ginsenosides, are hardly directly ionized by DART. This limited the application of DART to herbs with polar compounds as the main characteristics. The solution of this is to use auxiliary derivatization to increase their volatility. Even derivatization step is indispensable for obtaining the MS information of some polar compounds by DART. The steps, however, are much simpler compared to the conventional derivatization procedure. Extracts or solid sample contacted with the derivating agent in atmosphere enables derivatization of compounds which are lately ionized by DART [15].

DART is updating internally and externally in recent years to adapt itself to more accurate and stable qualitative and quantitative analysis. For example, more rigid and high throughput auto sampling module, isotope internal standard correction [8] and modified DART configuration with steeper ionization tip [49] have been successfully used in food analysis and other fields. Mass spectrometry is also developing quickly in recent years. Highly powerful hybrid mass spectrometer with both fragment information and accurate mass, ion mobility mass spectrometer with the capacity of separating isomers will be interestingly hyphenated with DART to work out components information in herbal medicine-related complex matrix.

Herbal medicines, especially for traditional Chinese medicines, with the property of complexity make sample pretreatments play an indispensable role in conventional analysis. DART-MS proposed a new chance for herbal medicine researches requiring minimal or even no sample pretreatments and LC separations, which extensively shortened the total analysis time and consumption. With the development of DART technique, more wide and deep applications of DART, e.g., high throughput analysis, rapid quantification, herbal medicine metabolite imaging and rapid illegal additive screening will be interestingly tried for efficient herbal medicine studies in the coming future.

REFERENCES

1. Takáts Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* 2004, 306(5695): 471–473.
2. Badu-Tawiah AK, Eberlin LS, Ouyang Z, Cooks RG. Chemical Aspects of the Extractive Methods of Ambient Ionization Mass Spectrometry. *Annual Review of Physical Chemistry* 2013, 64(1): 481–505.
3. Cody RB, Laramée JA, Nilles JM, Durst HD. Direct analysis in real time (DARTtm) mass spectrometry. *JEOL News* 2005, 40(1): 8–12.
4. Hajslova J, Cajka T, Vaclavik L. Challenging applications offered by direct analysis in real time (DART) in food-quality and safety analysis. *TrAC Trends in Analytical Chemistry* 2011, 30(2): 204–218.
5. Zhao Y, Lam M, Wu D, Mak R. Quantification of small molecules in plasma with direct analysis in real time tandem mass spectrometry, without sample preparation and liquid chromatographic separation. *Rapid Communications in Mass Spectrometry* 2008, 22(20): 3217–3224.
6. Nilles JM, Connell TR, Stokes ST, Durst HD. Explosives detection using direct analysis in real time (DART) mass spectrometry. *Propellants, Explosives, Pyrotechnics* 2010, 35(5): 446–451.
7. Duvivier WF, van Beek TA, Pennings EJ, Nielen MW. Rapid analysis of Δ-9-tetrahydrocannabinol in hair using direct analysis in real time ambient ionization orbitrap mass spectrometry. *Rapid Communications in Mass Spectrometry* 2014, 28(7): 682–690.
8. Busman M, Bobell JR, Maragos CM. Determination of the aflatoxin M1 (AFM1) from milk by direct analysis in real time-mass spectrometry (DART-MS). *Food Control* 2015, 47: 592–598.

9. Busman M, Maragos CM. Determination of T-2 and HT-2 toxins from maize by direct analysis in real time mass spectrometry. *World Mycotoxin Journal* 2015, 8(4): 1–10.
10. Lesiak AD, Cody RB, Dane AJ, Musah RA. Rapid detection by direct analysis in real time-mass spectrometry (DART-MS) of psychoactive plant drugs of abuse: The case of Mitragyna speciosa aka "Kratom". *Forensic Science International* 2014, 242: 210–218.
11. Wood JL, Steiner RR. Purification of pharmaceutical preparations using thin-layer chromatography to obtain mass spectra with Direct Analysis in Real Time and accurate mass spectrometry. *Drug Testing and Analysis* 2011, 3(6): 345–351.
12. Singh S, Verma SK. Study of the distribution profile of piperidine alkaloids in various parts of Prosopis juliflora by the application of Direct Analysis in Real Time Mass Spectrometry (DART-MS). *Natural Products and Bioprospecting* 2012, 2(5): 206–209.
13. Wang Y, Li C, Huang L, Liu L, Guo Y, Ma L, Liu S. Rapid identification of traditional Chinese herbal medicine by direct analysis in real time (DART) mass spectrometry. *Analytica Chimica Acta* 2014, 845(3): 70–76.
14. Zhou F, Zhu H, Liu S, Ma K, Song F, Liu ZQ. In Situ Analysis for Herbal Pieces of Aconitum Plants by Using Direct Analysis in Real Time Mass Spectrometry. *Chinese Journal of Chemistry* 2015, 33(2): 241–246.
15. Wang Y, Liu L, Ma L, Liu SY. Identification of saccharides by using direct analysis in real time (DART) mass spectrometry. *International Journal of Mass Spectrometry* 2014, 357(1): 51–57.
16. Rahman AFMM, Angawi RF, Kadi AA. Spatial localisation of curcumin and rapid screening of the chemical compositions of turmeric rhizomes (Curcuma longa Linn.) using Direct Analysis in Real Time-Mass Spectrometry (DART-MS). *Food Chemistry* 2015, 173(15): 489–494.
17. Chernetsova ES, Crawford EA, Shikov AN, Pozharitskaya ON, Makarov VG, Morlock GE. ID-CUBE direct analysis in real time high-resolution mass spectrometry and its capabilities in the identification of phenolic components from the green leaves of Bergenia crassifolia L. *Rapid Communications in Mass Spectrometry* 2012, 26 (11):1329–1337.
18. Bajpai V, Sharma D, Kumar B, Madhusudanan KP. Profiling of Piper betle Linn. cultivars by direct analysis in real time mass spectrometric technique. *Biomedical Chromatography* 2010, 24(12): 1283–1286.
19. Singh V, Gupta AK, Singh SP, Kumar A. Direct Analysis in Real Time by Mass Spectrometric Technique for Determining the Variation in Metabolite Profiles of Cinnamomum tamala Nees and Eberm Genotypes. *Scientific World Journal* 2012, 2012;1–6.
20. Singh A, Bajpai V, Srivastava M, Arya KR, Kumar B. Rapid screening and distribution of bioactive compounds in different parts of berberis petiolaris using direct analysis real time mass spectrometry. *Journal of Pharmaceutical Analysis* 2015, 5(5):332–335.
21. Li YJ, Wang ZZ, Bi YA, Ding G, Long SS, Musselman B, Zhang CF, Chen J, Xiao W. Direct analysis in real time ionization/quadrupole time-of-flight tandem mass spectrometry for rapid identification of iridoid glycosides and caffeoylquinic acids in Re Du Ning Injections. *Analytical Methods* 2013, 5(24):7081–7089.
22. Musah RA, Espinoza EO, Cody RB, Lesiak AD, Christensen ED, Moore HE, Maleknia S, Drijfhout FP. A High Throughput Ambient Mass Spectrometric Approach to Species Identification and Classification from Chemical Fingerprint Signatures. *Scientific Reports* 2015, 5: 11520.
23. Shen Y, van Beek TA, Claassen FW, Zuilhof H, Chen B, Nielen MW. Rapid control of Chinese star anise fruits and teas for neurotoxic anisatin by Direct Analysis in Real Time high resolution mass spectrometry. *Journal of Chromatography A* 2012, 1259(7): 179–186.
24. Fukuda E, Baba M, Iwasaki N, Uesawa Y, Arifuku K, Kamoe O, Tsubono K,Okada Y. Identification of Glycyrrhiza species by direct analysis in real time mass spectrometry. *Natural Product Communications* 2010, 5(11):1755–1758.
25. Kim HJ, Baek WS, Jang YP. Identification of ambiguous cubeb fruit by DART-MS-based fingerprinting combined with principal component analysis. *Food Chemistry* 2011, 129(3): 1305–1310.
26. Lee SM, Kim HJ, Jang YP. Chemometric Classification of Morphologically Similar Umbelliferae Medicinal Herbs by DART-TOF-MS Fingerprint. *Phytochemical Analysis* 2012, 23(5): 508–512.
27. Kim SW, Kim HJ, Kim JH, Kwon YK, Ahn MS, Jang YP, Liu JR. A rapid, simple method for the genetic discrimination of intact Arabidopsis thaliana mutant seeds using metabolic profiling by direct analysis in real-time mass spectrometry. *Plant Methods* 2011, 7(1): 1–10.
28. Lesiak AD, Cody RB, Dane AJ, Musah RA. Plant Seed Species Identification from Chemical Fingerprints—A High-Throughput Application of Direct Analysis in Real Time Mass Spectrometry. *Analytical Chemistry* 2015, 87(17): 8748–8757.
29. Cody RB, Dane AJ, Dawson-Andoh B, Adedipe EO, Nkansah K. Rapid classification of White Oak (Quercus alba) and Northern Red Oak (Quercus rubra) by using pyrolysis direct analysis in real time (DART™) and time-of-flight mass spectrometry. *Journal of Analytical and Applied Pyrolysis* 2012, 95(1): 134–137.
30. Kumar S, Bajpai V, Singh A, Bindu S, Srivastava M, Rameshkumar KB, Kumar B. Rapid fingerprinting of Rauwolfia species using direct analysis in real time mass spectrometry combined with principal component analysis for their discrimination. *Analytical Methods* 2015, 7(14): 6021–6026.
31. Kim HJ, Yong TS, Park SI, Jeong SH, Min KK, Jang YP. DART–TOF–MS based metabolomics study for the discrimination analysis of geographical origin of Angelica gigas roots collected from Korea and China. *Metabolomics* 2015, 11(1): 64–70.
32. Song Y, Liao J, Dong J, Chen L. Rapidly differentiating grape seeds from different sources based on characteristic fingerprints using direct analysis in real time coupled with time-of-flight mass spectrometry combined with chemometrics. *Journal of Separation Science* 2015, 38(17):3084–3089.
33. Fukuda E, Uesawa Y, Baba M, Suzuki R, Fukuda T, Shirataki Y, Okada Y. Identification of the country of growth of Sophora flavescens using direct analysis in real time mass spectrometry (DART-MS). *Natural Product Communications* 2014, 9(11): 1591–1594.
34. Zhu HB, Wang CY, Qi Y, Song FR, Liu ZQ, Liu SY. Rapid quality assessment of Radix Aconiti Preparata using direct analysis in real time mass spectrometry. *Analytica Chimica Acta* 2012, 752(8): 69–77.
35. Zeng S, Wang L, Chen T, Wang Y, Mo H, Qu H. Direct analysis in real time mass spectrometry and multivariate data analysis: a novel approach to rapid identification of analytical markers for quality control of traditional Chinese medicine preparation. *Analytica Chimica Acta* 2012, 733(6): 38–47.
36. Wang L, Zeng SS, Chen T, Qu H. Direct analysis in real time mass spectrometry, a process analytical technology tool for real-time process monitoring in botanical drug manufacturing. *Journal of Pharmaceutical and Biomedical Analysis* 2014, 91(5): 202–209.
37. Zeng SS, Chen T, Wang L, Qu HB. Monitoring batch-to-batch reproducibility using direct analysis in real time mass spectrometry and multivariate analysis: A case study on precipitation. *Journal of Pharmaceutical and Biomedical Analysis* 2013, 76(5): 87–95.
38. Zhou ZG, Zhang JL, Zhang W, Bai Y, Liu HW. Rapid screening for synthetic antidiabetic drug adulteration in herbal dietary supplements using direct analysis in real time mass spectrometry. *Analyst* 2011, 136(12): 2613–2618.
39. Li Y. Confined direct analysis in real time ion source and its applications in analysis of volatile organic compounds of Citrus limon (lemon) and Allium cepa (onion). *Rapid Communications in Mass Spectrometry* 2012, 26(10): 1194–1202.
40. Kim HJ, Jang YP. Direct analysis of curcumin in turmeric by DART-MS. *Phytochemical Analysis* 2009, 20(5): 372–377.
41. Xu B, Zhang DY, Liu ZY, Zhang Y, Liu L, Li L, Liu CC, Wu GH. Rapid determination of 1-deoxynojirimycin in Morus alba L. leaves by direct analysis in real time (DART) mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 2015, 114(10): 447–454.
42. Gao W, Qi LW, Liu CC, Wang R, Li P, Yang H. An improved method for the determination of 5-hydroxymethylfurfural in Shenfu injection by direct analysis in real time-quadrupole time-of-flight mass spectrometry. *Drug Testing and Analysis* 2015, 7(7):603–608.

43. Kim HJ, Oh MS, Hong Jk, Jang YP. Quantitative analysis of major dibenzocyclooctane lignans in schisandrae fructus by online TLC-DART-MS. *Phytochemical Analysis* 2011, 22(3): 258–262.
44. Doué M, Dervilly-Pinel G, Pouponneau K, Monteau F, Le Bizec B. Direct analysis in real time - high resolution mass spectrometry (DART-HRMS): a high throughput strategy for identification and quantification of anabolic steroid esters. *Drug Testing and Analysis* 2015, 7(7): 603–608.
45. Zeng SS, Wang L, Chen T, Qu HB. On-line coupling of macroporous resin column chromatography with direct analysis in real time mass spectrometry utilizing a surface flowing mode sample holder. *Analytica Chimica Acta* 2014, 811(6): 43–50.
46. Morlock GE, Ristivojevic P, Chernetsova ES. Combined multivariate data analysis of high-performance thin-layer chromatography fingerprints and direct analysis in real time mass spectra for profiling of natural products like propolis. *Journal of Chromatography A* 2014, 1328(7): 104–112.
47. Suh HW, Lee KB, Kim KS, Yang HJ, Choi EK, Shin MH, Park YS, Na YC, Ahn KS, Jang YP, Um JY, Jang HJ. A bitter herbal medicine Gentiana scabra root extract stimulates glucagon-like peptide-1 secretion and regulates blood glucose in db/db mouse. *Journal of Ethnopharmacology* 2015, 172(22): 219–226.
48. Shelley JT, Hieftje GM. Ionization matrix effects in plasma-based ambient mass spectrometry sources. *Journal of Analytical Atomic Spectrometry* 2010, 25(3): 345–350.
49. Häbe TT, Morlock GE. Quantitative surface scanning by Direct Analysis in Real Time mass spectrometry. *Rapid Communications in Mass Spectrometry* 2015, 29(6): 474–484.