Anal. Chem. **2010,** *82,* 8060–8070

Tracing Origins of Complex Pharmaceutical Preparations Using Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry

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A novel strategy to trace the origins of commercial pharmaceutical products has been developed based on the direct chemical profiling of the pharmaceutical products by surface desorption atmospheric pressure chemical ionization mass spectrometry (DAPCI-MS). Besides the unambiguous identification of active drug components, various compounds present in the matrixes are simultaneously detected without sample pretreatment, providing valuable information for drug quality control and origin differentiation. Four sources of commercial amoxicillin products made by different manufacturers have been successfully differentiated. This strategy has been extended to secerning six sources of Liuwei Dihuang Teapills, which are herbal medicine preparations with extremely complex matrixes. The photolysis status of chemical drug products and the inferior natural herd medicine products prepared with different processes (e.g., extra heating) were also screened using the method reported here. The limit of detection achieved in the MS/MS experiments was estimated to be 1 ng/g for amoxicillin inside the capsule product. Our experimental data demonstrate that DAPCI-MS is a useful tool for rapid pharmaceutical analysis, showing promising perspectives for tracking the entire pharmaceutical supply chain to prevent counterfeit intrusions.

It has been recently estimated by the World Health Organization (WHO) that approximately 5−8% pharmaceuticals (about US$ 75 billion globally in 2010[1]) on the market are counterfeits, raising a multibillion-dollar industry oriented problem worldwide. Accordingly, inferior pharmaceutical products could affect the health of millions of people and may lead to death in many cases.[1] Pharmaceutical analysis,[2−9] as a key step to prevent people from taking counterfeit pharmaceutical products, is thus of paramount importance. Huge efforts have been spent in the field of pharmaceutical quality analysis. Representative techniques including chromatography,[6,10,11] nuclear magnet resonance,[7,12] optical spectroscopy,[3,9] mass spectrometry,[2,13] ion mobility spectrometry,[4,11] thermal analytical methods,[14,15] various imaging techniques,[16−20] etc. have been employed for drug analysis. Generally, mass spectrometry-based methods[6,8,13,21,22] have been widely used, because of their excellent analytical performances such as high sensitivity, good specificity, high accuracy, wide applicability, and both qualitative and quantitative capability with reasonable precision. Traditionally, multiple-step sample pretreatment has to be performed before conventional mass spectrometric detection, thus greatly compromising the speed for pharmaceutical analysis. Recently, high throughput pharmaceutical analysis has been demonstrated using ambient ionization techniques such as de-

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10.1021/ac100407k
Published on Web 09/01/2010

sorption electrospray ionization (DESI),[19,23−28] direct analysis in real-time (DART),[29−33] surface desorption atmospheric pressure chemical ionization (DAPCI),[34−36] aerosol mass spectrometry (AMS),[2,37] atmospheric solids analysis probe (ASAP),[38] etc. These techniques require minimal or no sample pretreatment and enable direct, sensitive detection of multiple active ingredients in various pharmaceutical preparations such as tablets, liquids, and aerosol drugs. A single sample analysis can be completed within 1−10 s by ambient mass spectrometric methods, which renders ambient mass spectrometry as a preferred tool for sensitive and high throughput analysis of pharmaceutical products.

In the above-mentioned pharmaceutical analysis, most studies focused on the detection of active ingredients and/or impurities in drug preparations.[9,39−42] For example, multiple active drug compounds in Centre tablet were rapidly detected and identified using DESI-MS/MS experiments,[13] without sample pretreatment. Powdered pharmaceutical formulations were also rapidly analyzed using gasless DAPCI-MS,[34,35] with neither sample pretreatment nor chemical contamination, showing attractive applications of DACPI-MS/MS to rapidly detect active components of antibiotics on the market. However, pharmaceutical products usually contain much more chemical components rather than the active drug compounds. In such cases, the right dosage of the active ingredient itself cannot guarantee the safety of the drug preparation, which could be in any of the formulations such as capsules, powders, softgels, solutions, and aerosols. Thus, it is also a practically urgent need to obtain the full chemical profile of drug preparation for better safety.

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It is of increasing importance to safeguard the pharmaceutical supply chain, especially for chemical drugs produced via a multiple-step/location process or herbal medicine originated from natural plants, in which the components are likely dependent on both of the raw materials and the manufacturing procedures. Accompanied with drug adulteration, substandard pharmaceutical products sold on markets, pose serious damages to the manufacturer, the trader, and the customer. Normally, due to the matrixes, drug products from different manufacturers possess a unique molecular profile[42] regardless of the active components, which can be essentially the same. To improve the safety of the pharmaceutical supply chain, track-and-trace solutions based on chemical components and/or physical information are anxiously demanded.

The ideal technique for pharmaceutical analysis must possess the following merits: (1) capability to provide plenty of information, favorably at the molecular level; (2) highly sensitive detection of trace components in complex matrixes; (3) good resolution and high specificity; (4) capability for online monitoring in real time; (5) nondestructive analysis of drug-related materials without chemical contamination. The development of an easy-to-implement, "user-simple" system to track and trace merchandise from its origin/manufacturing location through the supply chain, regardless of its path, to the point-of-sale, is cost efficient and does not compromise production speeds of pharmaceutical industry. Certainly, ambient mass spectrometry becomes a choice for tracing the drug supply chain at the molecular level. Among the available ambient ionization techniques,[23–37] DAPCI can be operated without sheath gas for sensitive fine powder analysis or with sheath gas-assisted droplets for dried solid surface analysis, thus DAPCI-MS potentially meets most of the requirements of pharmaceutical analysis.

In this study, DAPCI-MS has been utilized to rapidly detect active components, degradation products and trace impurities in various commercial pharmaceutical preparations. Furthermore, the DAPCI mass spectral fingerprints are proposed as the molecular markers to trace the origins of pharmaceutical preparations with the same active components made by different manufacturers. This strategy has been further extended to analyze traditional Chinese medicine of complex matrixes.

EXPERIMENTAL SECTION

DAPCI-MS Analysis. All experiments were performed using a commercial linear ion trap mass spectrometer (LTQ-XL, Finnigan, San Jose, CA) coupled with a homemade DAPCI source. The DAPCI source and the LTQ mass spectrometer were set to work in either positive or negative ion detection mode. The pharmaceutical samples were supplied to the DAPCI source on a polytetrafluoroethylene (PTFE) plate, which was placed 2 to 3 mm below the discharge tip and centered horizontally between the ion entrance of the LTQ instrument and the DAPCI discharge needle. To avoid chemical contamination, only ambient air (~60% relative humidity) was used as the chemical reagent for the DAPCI process, without any other gas assistance. The DAPCI source is based on a four-dimensional automatic and precisely-controlled system (X, Y, Z, angle θ), which makes it more convenient to optimize the working conditions. Defined by the DACPI source coordinator system, the distance of the X-axis was set to be −8.0 cm, Y-axis was 3.5 cm, Z-axis was 3.0 cm, and the angle (θ) formed

Table 1. Information of Amoxicillin Preparations Tested

code	production location	specification (mg)	composition	color
1	Chengdu	500	amoxicillin, starch	slight yellow
2	Chengdu	500	amoxicillin, starch	white
3	Haerbin	250	amoxicillin, starch	slight yellow
4	Haikou	500	amoxicillin, starch	slight yellow
5 (counterfeit)	Unkown	250	starch, bitter agent	slight yellow

between the sample holder and the discharge needle was 45°. The discharge current was about 0.1 mA when the corona discharge voltage was ±3.5 kV. The temperature of the heated capillary of the LTQ instrument was maintained at 120 °C. Accelerated by the electric field, the reagent ions, which were primarily generated using humid ambient air, impacted the sample surface for desorption and chemical ionization such that the ions of analytes were created at ambient pressure and then introduced through the ion introduction system into the LTQ mass analyzer for mass analysis. Relatively low resolution (0.05 units) was used in this study, which potentially expands the method to wide applications where portable, cheap instruments of low resolution can be used instead of the expensive LTQ instrument. The mass spectra were recorded with an average duration time of about 30 s and background subtracted. Collision-induced dissociation (CID) experiments were performed by applying 18−30% collision energy (CE) to the precursor ions of interest, which were isolated using a mass-to-charge window with of 1.6 Da.

Materials and Reagents. Amoxicillin is one of the broad-spectrum β-lactam antibiotics of penicillin, which is widely used in the form of white crystalline powders. Four sources of amoxicillin capsules (Table 1) made by four different manufacturers A (500 mg), B (500 mg), C (250 mg), and D (500 mg) were bought from a local drugstore (Fuzhou, China). All of the amoxicillin passed authentication of Good Manufacturing Practice (GMP). The expired amoxicillin samples were prepared by continuously heating the normal samples from manufacturer A at 50 °C in darkness for 48 h. The counterfeit amoxicillin drug samples which contain no active drug component were obtained from a drug administration department. As certificated by the local drug administration department, the major component of the counterfeit drug was cheap starch qualified for only chemical industrial usages. Sometimes, talcum powder and bitter agents were also added to make counterfeits of amoxicillin, so that it was extremely difficult to differentiate the counterfeit amoxicillin from authentic preparation by the appearance and the taste of them. About 50 μL of water was nebulized onto a disposable PTFE plate to form a wet area (~1 cm^2), and the sample spot (~1 cm^2) was formed by covering the wet area using powdered dry amoxicillin (c.a., 20 mg). The PTFE plate with the drug sample was properly placed to the DAPCI source and then directly analyzed without further treatment.

Liuwei Dihuang Teapills (LDPs, also known as Six Flavor Rehmanni) are made of the six kinds of Chinese herbal medicines and have two formulas such as water-honeyed pills and concentrated pills, depending on their manufacturing procedures.[10] Briefly, with a certain proportion, fine powders of the six herbal medicines were homogeneously mixed at the first stage. For preparing the water-honeyed pills, gently boiled honey was added as the adhesive to the fine powders, allowing the production of bean-shaped LDPs. During the concentrated pill production, the extracts of the six herbal medicines became sticky fluids after low temperature boiling and then were used to glue the fine herbal medicine powders, resulting in LDPs for proper usages.

A total of 90 LDP samples including both water-honeyed pills and concentrated dry pills made by different manufacturers were purchased from local medical stores. The detail information is summarized in Table 2. Artificially degraded LDPs were prepared by air-bath heating the normal "Tongrentang" LDPs at 50 °C in darkness (~40% relative humidity, RH) for 6 h, by microwave irradiation for 10 min, or by exposure to humid air (~80% RH) for 48 h. All the LDP samples were analyzed after cutting them into halves without any chemical treatment. Only deionized water provided by the ECIT chemical facility was used as the ionization reagent.

Principal Component Analysis. Principal component analysis (PCA) of the mass spectral raw data (formatted into .txt files), using commercially available software STATISTIC (version 6.0, Statsoft, USA) was performed to visualize the differences between the pharmaceutical samples. The mass spectral data recorded with an averaged time of 20 s were exported to Excel software using the function "Export" of the Xcalibur software of the LTQ instrument. To ensure the accuracy of the data conversion, each m/z unit (e.g., m/z 80−81) was sampled by 15 data points. The m/z values were used as independent variables, and the signal intensities were used as dependent variables for all the text data. The matrix was loaded automatically, as a standard feature of the STATISTIC software, from the Excel file to the STATISTIC software. Once the data were successfully loaded, PCA was performed after normalization of the data.

RESULTS AND DISCUSSION

DAPCI-MS Detection of Chemical Drug Products. *DAPCI-MS of Powdered Amoxicillin Preparations.* As shown in Figure 1a, amoxicillin (MW 365) in the form of powdered sample (product A) was detected as the protonated molecules (m/z 366) and the sodiated molecules (m/z 388) under the positive ion detection mode. The protonated amoxicillin (m/z 366) generated major ionic fragments of m/z 349, 331, 303, and 286 (Figure 1b) in the CID experiment, probably due to the loss of NH_3, H_2O, CO, and OH,

Table 2. Information of LDP Preparations Tested

brand	production location	type	excipient	specification (g)	color
Tongrentang	Beijing	water-honeyed pill	honey	0.20	brownish black
999	Zaozhuang	water-honeyed pill	honey	0.25	brownish black
Xianhe	Dongying	water-honeyed pill	honey	0.20	brownish black
Jiuzhitang	Changsha	concentrated dry pills	no excipient	0.25	brownish black
Xiuzheng	Tonghua	concentrated dry pills	no excipient	0.20	brownish black
Zhongjing	Nanyang	concentrated dry pills	no excipient	0.20	brownish black

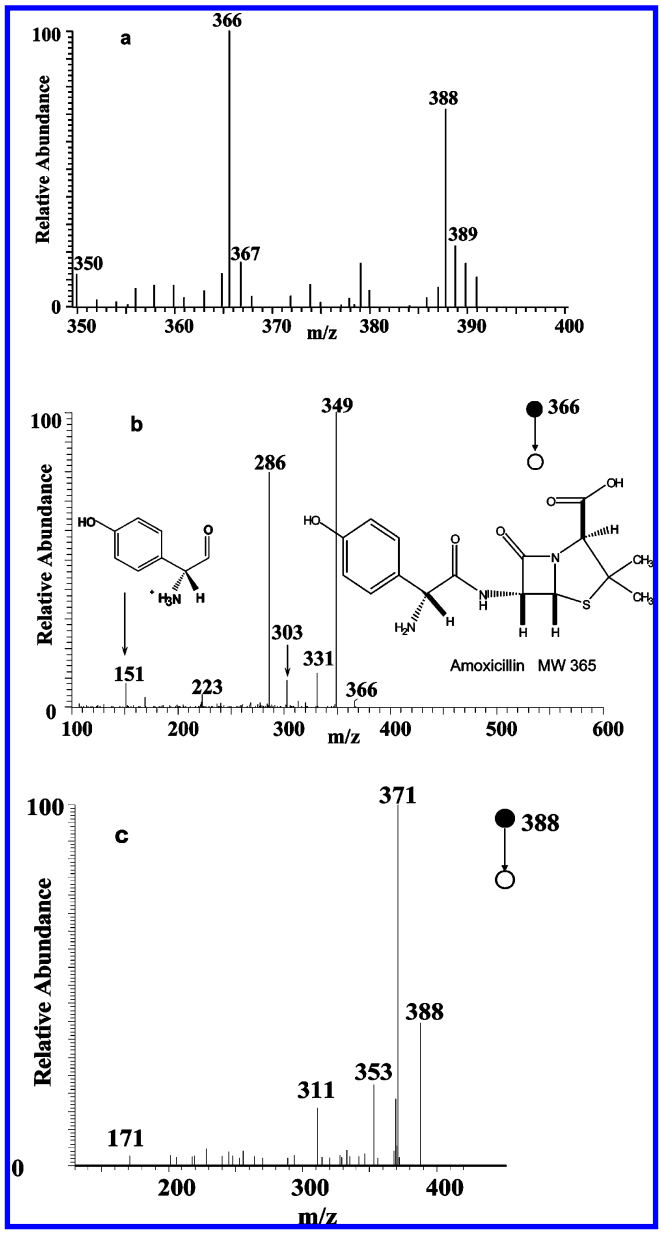

Figure 1. DAPCI mass spectra recorded from commercial amoxicillin capsules. (a) MS spectrum showing the protonated amoxicillin (*m/z* 366) and the sodiated amoxicillin (*m/z* 388); (b) MS/MS spectrum of the protonated amoxicillin (*m/z* 366); (c) MS/MS/MS spectrum of the sodiated amoxicillin (*m/z* 388).

successively. The fragment of *m/z* 349 was the base peak observed in the CID spectrum, because the energy required to break the NH_2-C bond was relatively low and the loss of ammonia easily occurred inside the ion trap under the CID conditions. The fragment of *m/z* 151 was ascribed to be the species of $(C_8H_9NO_2)^+$, which contained the benzene ring skeleton of the precursor molecule. In the MS^3 spectrum, the ions of *m/z* 349 generated major fragments of *m/z* 331 and 321 by the loss of water and CO, respectively. These fragments confirmed the successful detection of amoxicillin in the powdered sample. The sodiated molecules (*m/z* 388) generated major fragments of *m/z* 371, 353, and 311 (Figure 1c), due to the successive loss of NH_3, H_2O, and CH_2CO. These fragments indicate the amoxicillin molecule and its residues have high sodium affinity. The fragmentation pathways were confirmed using authentic amoxicillin compound using both DAPCI-MS and ESI-MS. Note that a predominant peak at *m/z* 349 was previously observed in the MS/MS spectrum, for which the precursor ions (*m/z* 366) were created using ESI.[43−47] Different small fragments of low intensities were also detected under different experimental conditions (e.g., ESI high voltage, collision energy, CID duration time).[43−47] The data indicated that the fragmentation of amoxicillin (*m/z* 366) was sensitive to the total energy deposited onto the ions. Generally, the DAPCI deposits slightly more energy than ESI to the protonated analyte.[48] Therefore, more fragments were detected from the DAPCI-MS/MS experiment. This feature might simplify the instrumentation of a mini mass spectrometer installed with a DAPCI source for amoxicillin detection, because significant fragmentation was observed, enhancing specificity and reducing the requirements for tandem MS experiments.

The limit of detection (LOD) of DAPCI-MS is generally low for many compounds tested.[34,35,49−53] In this study, the LOD of DAPCI-MS/MS measurements following amoxicillin fragments (*m/z* 349 and *m/z* 286) was estimated to be lower than 1 ng/g (S/N = 3, *n* = 16) in the drug preparation. Note that the LOD for amoxicillin without matrixes was much lower than 1 ng/g. A prominent signal of protonated amoxicillin was recorded from a dried amoxicillin spot (1 ppt, 10 μL) on a glass surface (Figure S1, Supporting Information). This result suggested that the matrixes in the amoxicillin preparation dramatically decreased the sensitivity of DAPCI, probably similar to the melamine case[50,53] where the analyte strongly binds to compounds of the matrixes. In most cases, the active drug compound is of a high concentration in the product, and no attempt was experimentally done to detect amoxicillin less than 1 ng/g in the powdered product.

DAPCI-MS for Differentiation of Chemical Drug Origins. Besides the active components, many signals ascribed to the chemicals in the matrix of the drug preparation were simultaneously detected in the DAPCI-MS spectrum. For example, the abundant signals observed at *m/z* 88, 175, 212, etc. (Figure 2a) were irrelative to the amoxicillin molecules in the drug product A. Although the amoxicillin was detected as the protonated molecule (*m/z* 366) and the sodiated molecule (*m/z* 388), the relative intensity of the sodiated peak was 7 times higher than that of the protonated peak. This was accounted for by the high content of sodium in preparation A. This suggestes that the mass spectral fingerprints of the drug products might be able to differentiate the product with different origins.

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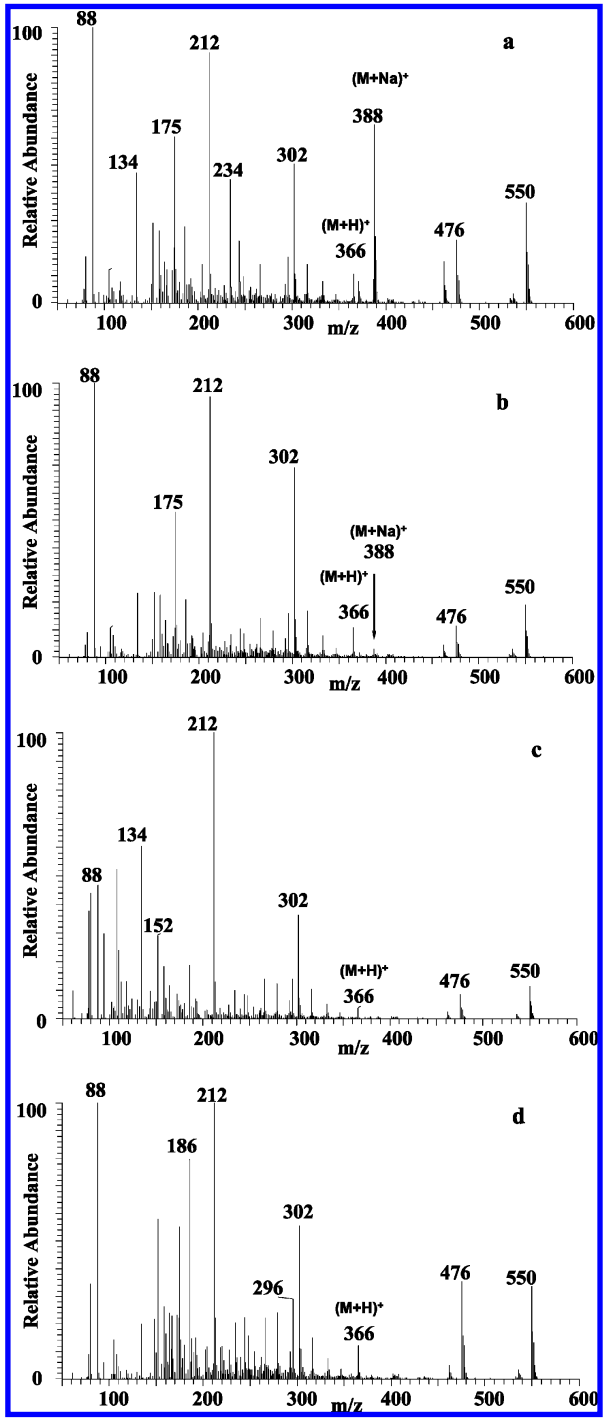

Figure 2. DAPCI mass spectral fingerprints recorded from four sources of amoxicillin capsules made by different manufacturers (A, B, C, D). (a) MS spectrum recorded from capsule A; (b) MS spectrum recorded from capsule B; (c) MS spectrum recorded from capsule C; (d) MS spectrum recorded from capsule D.

As shown in Figure 2b−d, three other amoxicillin products, B, C, and D, manufactured by different plants give differentiable mass spectral fingerprints in the mass range of 50−600 Da. In these spectra, the active components of amoxicillin were detected at m/z 366 for all four products. The signal levels of m/z 366 detected in product A, B, and D were almost the same (1.3×10^5 cps), showing an abundance of 2 times higher than the one detected in product C (6.2×10^4 cps). Besides the protonated amoxicillin signals, many other signals were detected from each

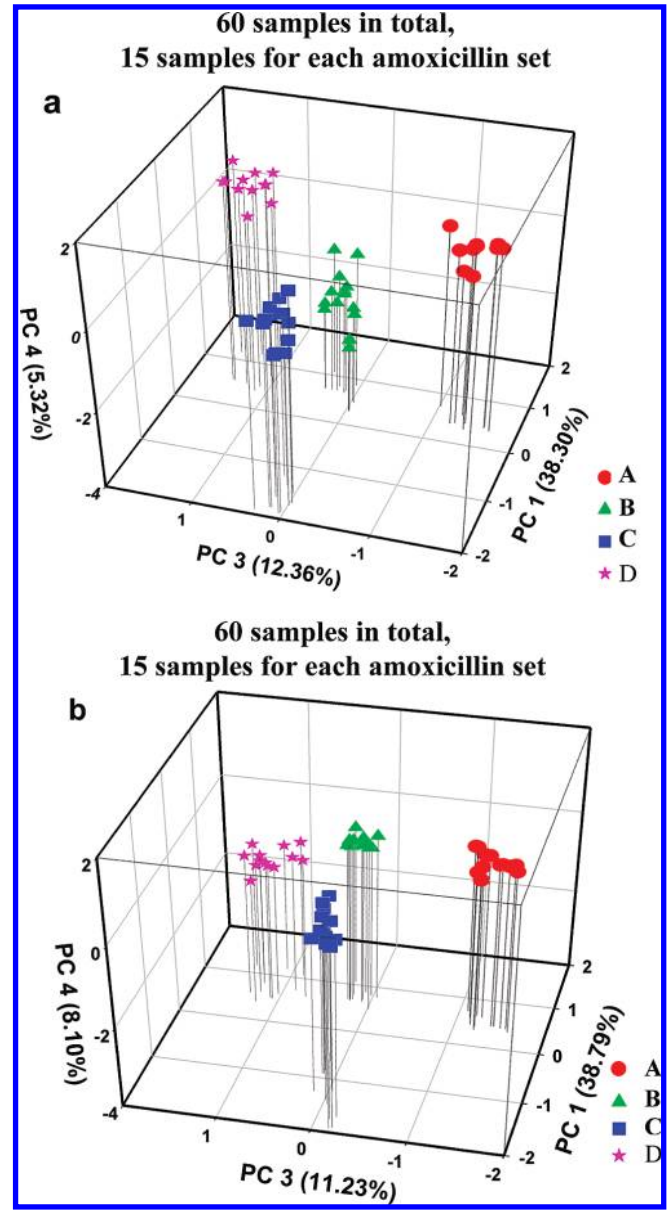

Figure 3. Differentiation of four sources of commercial amoxicillin capsules using DAPCI mass spectral fingerprints. (a) 3-D PCA score plots achieved using the mass spectral data recorded in the range of m/z 50−600; (b) supervised PCA score plots using the mass spectral data recorded in the range of m/z 50−600 without the signals (i.e., m/z 366, 388) of amoxicillin.

product, with varied signal intensities. For instance, the sodiated peak at m/z 388 was the most abundant in the product A but almost undetectable in the products B, C, and D. These data confirmed that the difference in matrixes such as sodium content in the products was detectable by DAPCI-MS.

The mass spectral data were then subjected to PCA, which resulted in well separated clusters in the 3-D PCA score plots (Figure 3a). As noted in Figure 2, the active component (i.e., amoxicillin) of product C differed from the other products. This aspect is also shown in Figure 3a, where the product C is located far from the other products at the axis of PC1. Alternatively, supervised PCA was also performed using the same mass spectral data without the peaks at m/z 366 and m/z 388. As expected, the pattern recognition was successfully achieved with tight clusters (Figure 3b). In comparison with Figure 3a, the samples were even

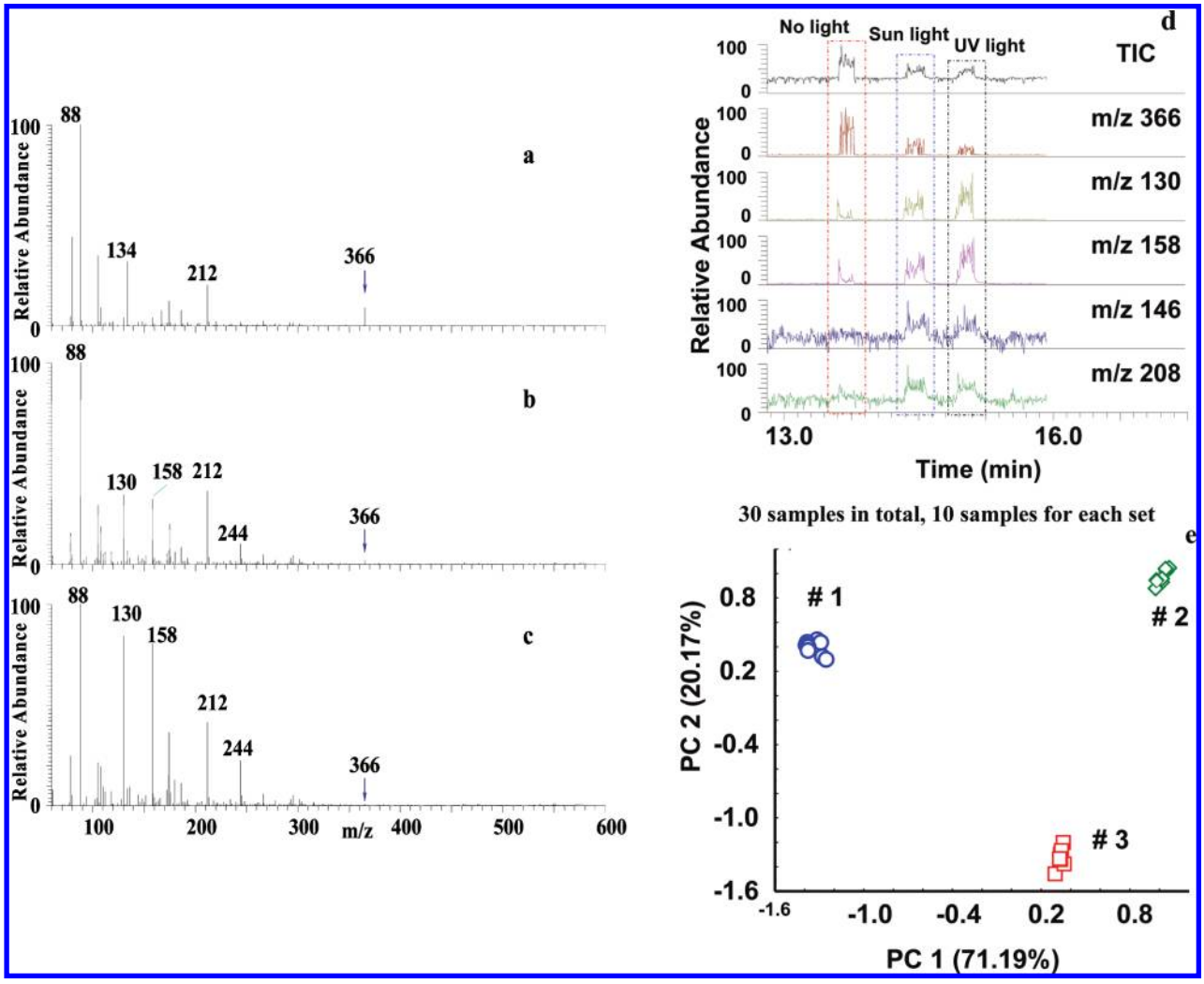

Figure 4. DAPCI-MS for rapid detection of inferior amoxicillin products. The DAPCI-MS fingerprints recorded from amoxicillin products exposed to no light (a), to sunlight for 4 h (b) and to UV light (260 nm) for 4 h (c); (d) signal traces of different components (*m/z* 366, 130, 158, 146, and 208) in the amoxicillin products subjected to no light exposure (left panel), 4 h sunlight exposure (middle panel), and 4 h UV light (260 nm) exposure (right panel); (e) PCA score plots obtained using the DAPCI mass spectral fingerprints recorded from the amoxicillin products subjected to no light exposure (# 1), 4 h sunlight exposure (# 2), and 4 h UV light (260 nm) exposure (# 3).

more tightly clustered in Figure 3b, probably because the varying signals at *m/z* 366 and *m/z* 388 detected from different individual samples of the same type products (i.e, A, B, C, and D) were eliminated. On the other hand, the tight clusters proved that the mass spectra of each product were reproducibly recorded using DAPCI-MS. These data show that DAPCI-MS spectral fingerprints can be used to differentiate the origins of the chemically prepared drug products.

DAPCI-MS Detection of Drug Degradation. Amoxicillin is sensitive to UV light irradiation so that it is suggested to be kept in darkness at room temperature. The light-induced degradation usually results in decreased concentration of the active components and elevated levels of side products, which could be potentially toxic to patients. However, as we tested, the inferior drug amoxicillin preparations could not be recognized by a sensory method, even when the powdered drug was exposed to sunlight for 4 h. Alternatively, DAPCI-MS is proposed as a useful tool to detect the partly degraded amoxicillin preparations. As shown in Figure 4, the mass spectral fingerprints recorded from the same amoxicillin product before light exposure (Figure 4a) and after exposure to sunlight for 4 h (Figure 4b) or to the UV light of a lamp (260 nm, 5 W) for 4 h (Figure 4c) differed from each other. For example, the signal of amoxicillin (*m/z* 366) decreased down to 40% and less than 20% after the 4 h sunlight exposure and the 4 h UV light irradiation, respectively (Figure 4d). Meanwhile, the peak at *m/z* 158, which was almost undetectable in the original drug product, was abundantly detected after sunlight exposure; this signal was further enhanced for the case of the same drug product irradiated under the UV light, showing very good correlations with the light degradation process. Upon CID, the ions of *m/z* 158 generated major peaks at *m/z* 143, 130, 116, and 88, probably by the loss of CH_3, CO, $CH_2{=}CHCH_3$, and $[CO{+}CH_2{=}CHCH_3]$, respectively. Thus, the precursor ions (*m/z* 158) were tentatively assigned to $(C_6H_8O_2NS)^+$, which was produced by the symmetric cleavage of the four-member ring of the amoxicillin molecule at the N−CO and the SC−CNH bonds. Simultaneous photocleavage of two bonds requires more energy and possibly produces small species by further fragmentation. Accordingly, the most abundant fragment of *m/z* 130 observed in the MS^2 spectrum of the $(C_6H_8O_2NS)^+$ ions

(m/z 158) should be detectable from the MS spectrum recorded from the samples after light exposure. In fact, as shown in Figure 4d, the total ion current (TIC) trace of the signal detected at m/z 130 was well correlated with the light dosage applied for irradiation. In addition, the signal intensities of peaks at m/z 146 and 208 were enhanced after light exposure. These two peaks were ascribed to the product ions generated by the breakage of the OCN−CCN bond in the amoxicillin molecule and by the neutral loss of H_2O and CO_2 during the photoinduced degradation process. These peaks were also detected as the degradation products of amoxicillin in the solution phase.[43,54] However, the peaks at m/z 158 and 130 were not reported for amoxicillin degradation in solution. This indicated that the photodissociation path differed from those observed in aqueous solution[43,45,46,55−58] or in biological processes,[59−61] probably because the photolysis occurred on the solid surface without the significant influences of water. The exact mechanism could be illustrated only with extra effort, which is beyond the scope of this study.

Principal component analysis was also performed to visualize the difference between the samples at different photolysis stages. Figure 4e shows that all the samples at different stages are separated in the PCA score plots, where the samples exposed to the sunlight were located between the natural amoxicillin products and those exposed to the UV light. The PCA results demonstrated that DAPCI-MS can separate the different stages of the photolysis of amoxicillin. These findings also suggest that the matrixes of the pharmaceutical preparations provide useful information to differentiate the quality of the product, especially the possible degree of degradation of active compounds.

DAPCI-MS Detection of Drug Counterfeits. Due to the wide applicability, low-quality antibiotics such as amoxicillin can be found on the market. To test the feasibility of DAPCI-MS for detection of drug counterfeits, experiments were performed to differentiate the normal amoxicillin preparations from the expired drugs and the counterfeits. For the purpose of extra profits, the counterfeits usually contain cheap filling agents (e.g., starch) and no/much less active component. The composition of the expired drugs differs from the unexpired ones, but the ingredients vary in a wide range, depending on the storage time and conditions. Although it is hard to tell by the sensory methods, the difference between the chemical compositions of the normal drug preparations, the expired ones, and the counterfeits were easily detected by DAPCI-MS (as shown in Figure S2, Supporting Information). The differentiation is visualized by the PCA score plots (Figure 5a) obtained using the mass spectral fingerprints of all 90 samples. The data clearly demonstrate the technique developed here is able to rapidly screen inferior amoxicillin preparations from the normal ones.

The reproducibility of the measurements was also experimentally evaluated using 90 normal amoxicillin preparations, which were randomly selected from three batches of products made by the same manufacturer (B). Figure 5b shows the PCA score plots of three sets of samples, showing no separation between these samples, because the samples tested were chemically similar to each other. Once another set of samples made by a different manufacturer were added into the data set for PCA analysis, the total samples were separated according to the manufacturers rather than the batch numbers (Figure 5c). This also supports that, as long as they are produced by the same manufacturer (i.e., the same procedure and materials), the chemical difference between the drug preparations of different batch numbers is almost negligible.

To test the long-term stability of the method, experiments were run on different dates using the samples (different individual amoxicillin capsules) produced by the same manufacturer and of the same batch number. As the result, Figure 5d shows the PCA score plots obtained using the mass spectral fingerprints recorded at day 1, day 3, and day 6. Although the data points collected in the sixth day are located a bit far from the first day, there is no significant space separating the sample sets analyzed on different dates. Once another data set was recorded from samples made by different manufacturers, the data points shown in Figure 5d were tight clusters in the PCA score plots (data not shown). Alternatively, the deviation of the TIC of all the mass spectra recorded on different dates was 14.6% (Table S1, Supporting Information), which was reasonable for trace analysis using mass spectrometry without sample pretreatment. Therefore, these data demonstrate that DAPCI-MS provides reasonable reproducibility for fast drug analysis.

DAPCI-MS Detection of Herbal Medicine Products. Pharmaceutical products originated from natural plants are of increasing interest. A large amount of herbal medicine preparations have been widely used in different countries. Liuwei Dihuang Teapill (LDP), one of the most representational traditional Chinese medicines (TCMs) from Song dynasty, provides reliable effects to cure diseases of immune and endocrine systems such as the kidney-yin (kidney function deficient), diabetes, night sweat, lumbago, etc.[62,63] LDP is depurated from six natural plants containing active components such as radix rehmanniae preparata, rhizoma dioscoreae, fructus corni, cortex moutan, etc. Therefore, LDP plays a role which any single component cannot do in the human body. The final quality of the LDP is highly dependent on the raw materials of the natural plants and also the procedure used in the production chain. Being a complex mixture, LDP challenges analytical tools for its quality control, which is qualitatively done using sensory methods (e.g., based on the smell, taste, physical appearance, etc.) to provide trustworthy results or by sensitive instrumental methods such as micellar electrokinetic capillary chromatography (MEKC),[64] infrared spectrum analysis,[65]

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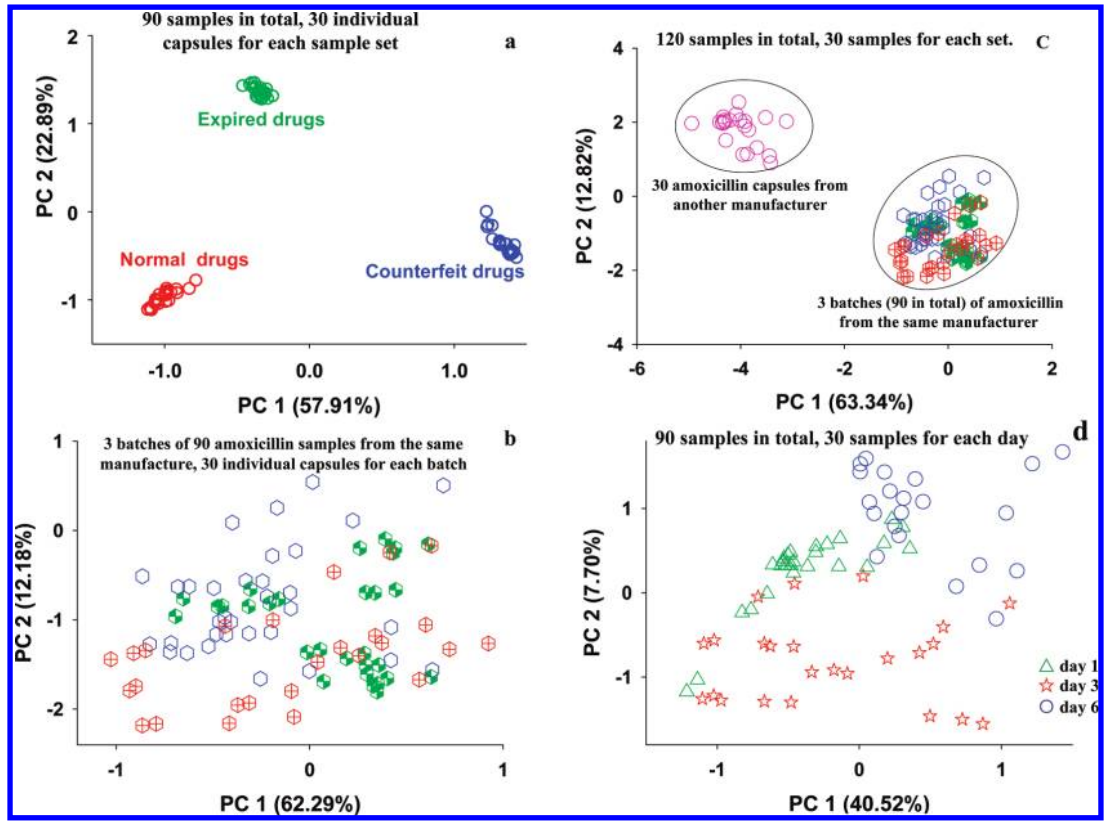

Figure 5. Differentiation of the overall quality of different amoxicillin products. (a) PCA score plots obtained using the DAPCI fingerprints of the normal, expired, and the counterfeit amoxicillin preparations; (b) PCA score plots obtained using the DAPCI fingerprints of three batches of amoxicillin made by the same manufacturer; (c) PCA score plots of three batches of amoxicillin from the same manufacturer and amoxicillin products from another manufacturer; (d) PCA score plots of amoxicillin obtained using the mass spectral fingerprints recorded at day 1, day 3, and day 6.

X-ray diffraction analysis,[66] and HPLC/HPLC-MS,[67−70] without sample pretreatment. Therefore, LDP was selected as the representative sample for this study to validate the strategy using DAPCI-MS to trace the origins of commercial LDP products.

Detection of Active Components in Herbal Medicine Products. As reported previously,[62,68,71] the major fraction of the LDP active components are acidic compounds including radix rehmanniae preparata, rhizoma dioscoreae, fructus corni, and cortex moutan. As shown in Figure 6a, these compounds were easily deprotonated to show their signals in the negative mode DAPCI mass spectrum. Upon CID (22% CE, 30 ms), the deprotonated gallic acid (m/z 169) generated ions of m/z 151, 141, and 125 as the major fragments (Figure 6b), by the loss of H_2O, CO, and CO_2, respectively. The negatively charged paeonol molecules (m/z 165) yielded fragments of m/z 149, 147, and 121 (Figure 6c) under the CID conditions by the loss of CH_4, H_2O, and C_2H_2O, respectively. The aucubin (m/z 345) produced fragments of m/z 327, 309, and 301 (Figure 6d) under the CID conditions by the loss of H_2O, $2H_2O$, and CO_2, respectively. The ursolic acid (m/z 455) produced ions of m/z 437 and 357 (Figure 6e) as the major fragments by the loss of H_2O and $C_6H_{10}O$, respectively. These assignments were confirmed using authentic compounds with the CID data matching. These data show DAPCI-MS is able to detect the active components of pharmaceutical preparations originated from natural plants.

As shown in Figure S3 (Supporting Information), the LDP products provide similar mass spectral fingerprints recorded using negative DAPCI, especially in the range of m/z 50−200. On the basis of these fingerprints, the six sources of LDPs were not well separated in the PCA score plots (Figure S4, Supporting Information). This was because all the LDP samples were commercially available products, which should pass the quality examination before they were allowed to enter the market. Usually, only the active compounds (i.e., acidic chemicals in LDPs) were examined for the quality control purpose; thus, the content of these compounds should be assured although the samples were produced by different manufacturers. Thus, it was not surprising to see a very similar spectral pattern of different LDPs under the negative ion detection mode.

DAPCI-MS Fingerprint Profiling of Herbal Medicine Products. Positive DAPCI experiments provided abundant signals in a wide mass range of m/z 50−350. Note that the effective components of LDP were acidic compounds, which were

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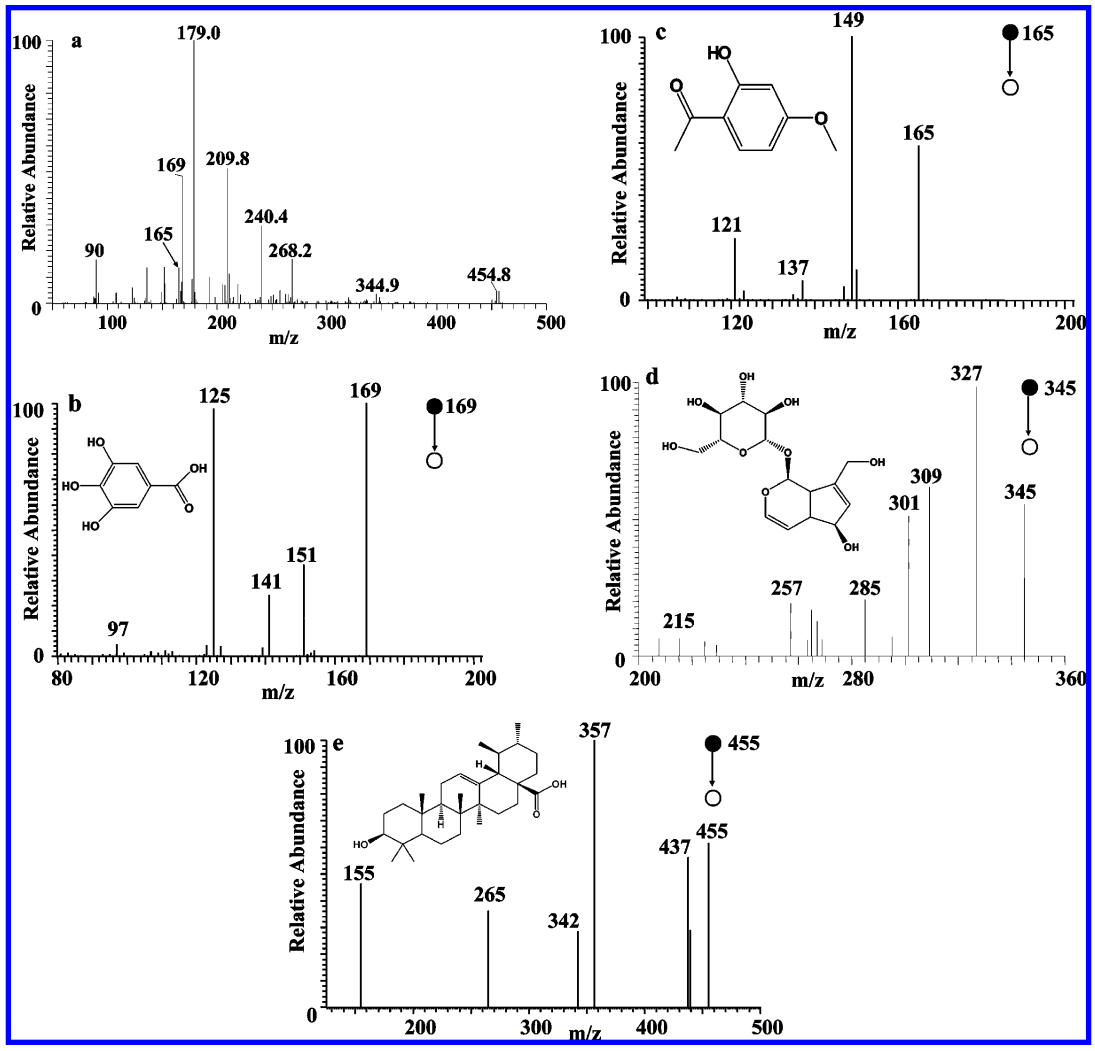

Figure 6. DAPCI-MS for rapid detection of active components in LDP under the negative ion detection mode. (a) DAPCI mass spectrum of LDP; (b) MS/MS spectrum of deprotonated gallic acid; (c) MS/MS spectrum of deprotonated paeonol; (d) MS/MS spectrum of deprotonated aucubin (*m/z* 345); (e) MS/MS spectrum of deprotonated ursolic acid (*m/z* 445).

unlikely to be detected in the positive ion detection mode; the signals recorded in positive DAPCI measurements mostly reflected the chemical information of the matrixes. Therefore, each of these six sources of LDPs generated unique mass spectral fingerprints (Figure 7a–f) although these samples were qualified commercial products. As mentioned above, these peaks, which were irrelative to the active components of the LDPs, were most likely originated from the raw materials and processing technology. For example, 5-hydroxymethyl-furfural (5-HMF), one of the pyrolysis products of glucose, widespread in the LDPs,[71] was detected as the protonated molecules at *m/z* 127, which produced major product ions of *m/z* 109, 99, and 85 by the loss of water, CO, and CH_2CO, respectively (inset of Figure 6a). The ionic fragment of *m/z* 99 may cleave water, yielding a small signal at *m/z* 81 in the CID spectrum. Note that no 5-HMF was directly detected from the original "Xianhe" LDP samples, but the signal of 5-HMF (*m/z* 127) appeared from the "Xianhe" LDPs heated at 120 °C for 30 min. This indicates that the high temperature heating process should be avoided during the production process of the "Xianhe" LDPs.

The chemical fingerprints of 90 samples from six sources of LDPs acquired using positive DAPCI-MS at the range of *m/z* 50–350 were subjected to principal component analysis. Six sources of the LDP samples were clustered into six categories (Figure 8a) according to the different manufacturers, showing the differential origins of the qualified LDPs. The distance between the clusters of the sample "Tong Ren Tang" (honey pill, spot 1) and "Zhong Jing" (concentrated pill, spot 6) is much shorter than that among other samples, possibly because the two sources of LDPs were more chemically similar than the others. For the same type of the LDPs (i.e., from the same manufacturer), the LDPs produced on different dates were not separated in the PCA score plots. Note that the active components were almost at the same levels for all the LDP commercial samples. Thus, the separation of these six sources of LDPs was attributed to the matrixes of the natural plants and the possible materials involved in the production processes. More interestingly, the honeyed water pills (three types, 45 samples) and the concentrated dry pills (three types, 45 samples) were separated into two clusters in the 3-D PCA score plots. This further suggests that the variances occurring in the manufacturing process provide no alteration of the quality (in terms of active component) of the drug products but useful information to trace back their processing origins.

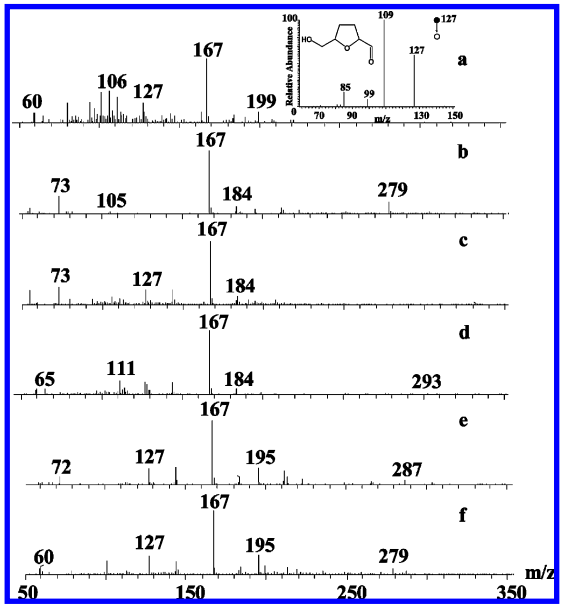

Figure 7. Chemical fingerprints of six sources of LDPs recorded in the positive ion detection mode of DAPCI-MS. (a) "Tong Ren Tang"; (b) "Xian He"; (c) "999"; (d) "Jiu Zhi Tang"; (e) "Xiu Zheng"; (f) "Zhong Jing".

To further validate this hypothesis, 15 DAPCI mass spectra (representatively shown in Figure S5, Supporting Information) recorded from "Tongren Tang" LDPs heated at 120 °C for 5 min were included in the data set to run PCA again. As shown in Figure 8b, the separation pattern obtained for the six sources of LDPs was not changed, but the heated samples were well separated from the normal commercial products. The tight clusters achieved indicated that the precision of the mass spectrometric measurement was good enough for the differentiation purpose. Therefore, these data confirmed that a different processing procedure such as extra heating could be revealed using the DAPCI mass spectral fingerprints directly obtained without sample pretreatment.

Extra experiments were performed to examine the applicability of DAPCI-MS for fast screening inferior herbal medicines from the normal ones. Figure 8c shows the PCA score plots obtained using the data set recorded from the same manufacturer but subjected to different conditions. The intensively heated samples (using a microwave) are far separated from the normal LDPs in PC1 rather than in PC2. Interestingly, the moderately heated samples (50 °C, using air bath) are located in the middle of the space (PC1) between the normal samples and the most heated ones. The PC2 levels for the two heated sample sets are relatively close (−1.2 to −1.5). However, the samples exposed to humid air are separated from the normal ones in both PC1 and PC2, showing the dramatic difference between these samples caused by the treating processes. The separation pattern obtained using these samples confirms that DAPCI-MS combining PCA for data processing is a valuable tool to visualize the difference of herbal medicine preparations associated with the production process (e.g., difference in raw materials and/or manufacturing procedures), even for the preparations with complex matrixes such as LDPs.

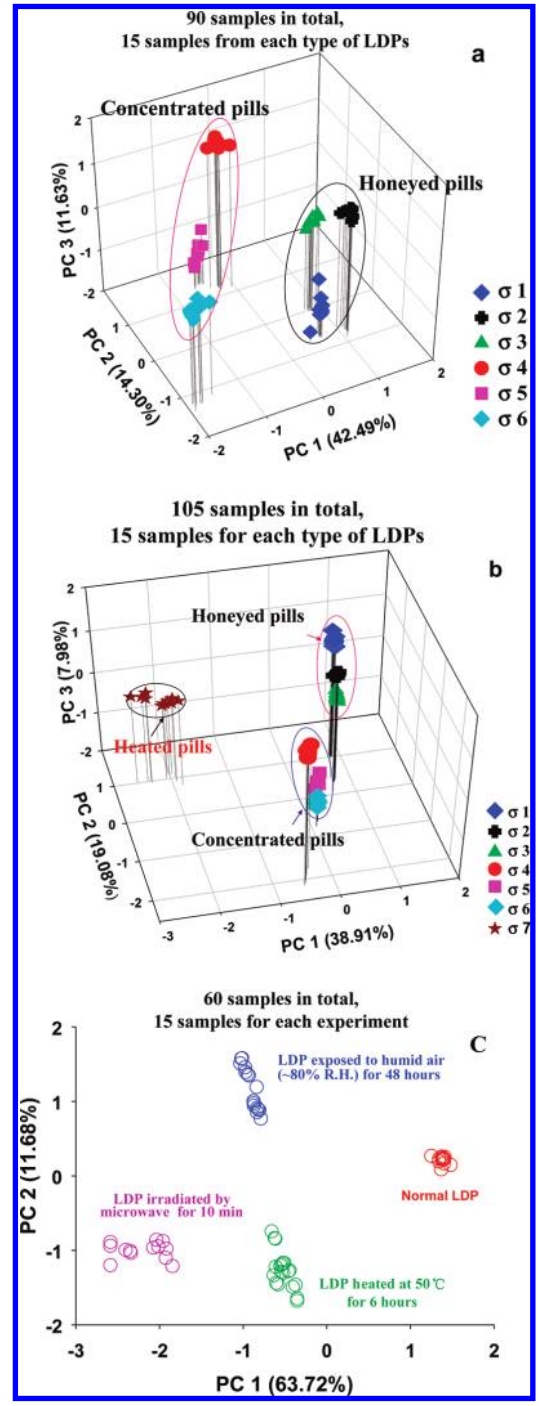

Figure 8. Differentiation of the overall quality and original manufacturers of different LDP products. (a) 3D-PCA score plots obtained using the DAPCI-MS fingerprints of six sources of LDPs; (b) 3D-PCA score plots obtained using the DAPCI-MS fingerprints of six sources of LDPs plus one set of samples under an extra heating process at 120 °C for 5 min. (σ1) "Tong Ren Tang", (σ2) "Xian He", (σ3) "999", (σ4) "Jiu Zhi Tang", (σ5) "Xiu Zheng", (σ6) "Zhong Jing", (σ7) "Tong Ren Tang" heated for 5 min; (c) PCA score plots obtained using the data set recorded from the same manufacturer ("Tong Ren Tang") but subjected to different treating processes.

Analysis Speed. Generally, DAPCI mass analysis, even tandem mass analysis, can be done within seconds.[72] However, the sample pretreatment process takes time, particularly for

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samples with complex matrixes. In this study, 90 samples (i.e., 900 measurements) were completed within 900 min, with 1 min of the average time for each sample measurement (including the time for sample loading). In comparison with HPLC-MS, the measurement time has been greatly reduced due to the minimal requirements for the sample pretreatment. This shows attractive features of DAPCI-MS with the real-world capability for high-throughput analysis of complex samples in the pharmaceutical industry.

CONCLUSIONS

The counterfeit "industry", especially the pharmaceutical industry, has become a global problem. Being a useful tool for rapid analysis of complex samples without any chemical contamination, DAPCI-MS is proposed as the alternative technique to differentiate the origins of pharmaceutical products. As demonstrated, DAPCI-MS is able to detect the active components of the drug preparations and a good variety of the matrix compounds irrelevant to the drug compounds, with high throughput. More importantly, the data show that the chemical fingerprints of the drug matrixes provide valuable information, which is useful to track the origins of the pharmaceutical preparations or to differentiate the overall quality of the pharmaceutical products. This strategy has been validated using both antibiotics such as amoxicillin and traditional Chinese medicine originated from natural plants. Thus, the experimental data prove that DAPCI-MS is a valuable tool for the entire pharmaceutical supply chain to prevent counterfeit intrusions and for rapidly differentiating the quality of commercial pharmaceutical products.

ACKNOWLEDGMENT

This work was supported by the Innovation Method Fund of China (2008IM040400) and a grant from MOST of China (No. 2009DFA30800).

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review February 14, 2010. Accepted August 17, 2010.

AC100407K