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ORIGINAL PAPER

Rapid screening of active ingredients in drugs by mass spectrometry with low-temperature plasma probe

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Abstract A high-throughput method for rapid screening of active ingredients in drugs has been developed with mass spectrometry coupled to a low-temperature plasma (LTP) probe ion source. Without sample preparation or pretreatment, the active ingredients of 11 types of commercial pharmaceuticals, including hormones, antipyretic analgesics, cardiovascular, digestant, neuro-psychotherapeutic, diuretic, antithyroid, sulfa anti-inflammatory, antiparastic, sedative-hypnotics, and antibacterial, were directly desorbed/ionized and detected by a linear ion trap mass spectrometry (MS). The structures of these ingredients were elucidated by tandem MS. The analysis of 18 methyltestosterone tablets could be accomplished within 1.9 min, which allows fast detection with a speed of approximate 600 samples within 1 h. This work demonstrated that LTP probe ion source combined with MS is a high-throughput method for screening of pharmaceuticals and potentially applied to on-line quality control in pharmaceutical industry.

Keywords Rapid screening · LTP probe · Drugs · Mass spectrometry · Ambient ionization

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Introduction

Drug discovery-based technologies that involve parallel and combinatorial chemistry synthesis have led to an increase in the number of samples requiring analysis [1]. The speed of analyzing these compounds for structural confirmation then becomes the limiting factor in the drug development process [2]. Mass spectrometry (MS)-based analytical techniques are highly expected for drug screening in pharmaceutical industry because of its high sensitivity, short analysis time, and capability of structural elucidation gained through employing MS/MS by using triple quadrupoles, ion trap, or quadrupole time-of-flight mass spectrometers [3–5]. However, sample preparation, extraction, and chemical derivatization steps of present MS method are often necessary to identify active ingredients in drugs [6, 7].

In recent years, several ambient desorption/ionization methods have been developed for direct surface analysis of solid samples with little or no sample pretreatment [8–10]. These methods mainly include desorption electrospray ionization (DESI) [11–13], direct analysis in real time (DART) [14], desorption atmospheric-pressure chemical ionization (DAPCI) [15], plasma-assisted desorption/ionization [16], and dielectric barrier discharge ionization (DBDI) [17, 18]. Analytes consist of drug tablets [16, 19], explosives [18, 20], metabolites [21], and biological molecules [11], which were deposited or mounted on a large variety of surfaces. Recently, a low-temperature plasma (LTP) probe ion source was proposed by Cooks group who collaborated with our group, which allows direct interaction of the plasma with the sample without having to place it between two electrodes [22].

In the present work, LTP probe is explored as ion source for the rapid screening of the pharmaceutical samples with MS. Eleven types of selected commercial drugs were

examined, and approximately 600 samples per hour were analyzed without any sample pretreatment. The present work provides a high-throughput method for rapid screening of active ingredients in pharmaceuticals research and would be potentially applied as a useful tool for drug quality control.

Experimental section

Instrumentation

The commercial Finnigan LTQ ion trap mass spectrometer (Thermo Electron Corp., San Jose, CA, USA) was used throughout all experiments. Mass spectra were processed using the instrument software Interface (Xcalibur version 1.4 SR1). The mass spectrometry conditions were as follows: tube lens voltage 95 or −95 V; heated capillary voltage 33 or −33 V; capillary temperature 275°C; multiplier voltages 1 and 2, −1,200 V; multipole 00 offset −4.0 V; intermultipole lens 0 voltage −4.20 V; multipole 0 offset −4.5 V; intermultipole lens 1 voltage −15 V; gate lens voltage −40 V; multipole 1 offset −8.0 V; front lens −5.25 V; and multipole r.f. amplitude ($V_{p\text{-}p}$) 400 V. Discharge powers of 5–30 W with a peak-to-peak voltage of 3.5–4.5 kV and a frequency of 18.0–25.0 kHz were purchased from Beili Guoke Co. Ltd. (Beijing, China).

Reagents

All over-the-counter and prescription drugs were obtained from the local pharmacies. Eleven types of pharmaceuticals (total number of 22 samples) based on pharmacological classification have been studied in the present study, including (a) hormones (steroidal), (b) antipyretic analgesics (nonsteroidal anti-inflammatory), (c) cardiovascular, (d) digestive system, (e) neuro-psychotherapeutic, (f) diuretic, (g) antithyroid, (h) sulfa anti-inflammatory drugs, (i) antiparastic, (j) sedative-hypnotics, and (k) antibacterial. Granules, capsules, and tablets investigated are listed in Electronic supplementary material (Figure S-1). Helium (99.99%), nitrogen (99.99%), and argon (99.99%) from Huayuan Gas (Beijing, China) were used as the discharge gases. Uncoated tablets were analyzed directly, while the protective films of coated tablets were carefully scraped to expose the active ingredients before analysis. Drug powder sealed in granules and capsules, such as nimesulide and flunarizine, could be analyzed after exposure without compression.

Fabrication of LTP probe

The LTP probe was similar to that used in our previous report [23], which consisting of a quartz tube (O.D. 3.0 mm and I.D. 1.8 mm) with an internal electrode (copper rod with diameter of 1.5 mm) centered axially and an outer electrode (copper paper) wrapped on the outer wall of the tube (Fig. 1). An alternating current voltage was applied to generate atmospheric-pressure plasma. The discharge gases including helium, argon, and nitrogen were introduced from the top arm into the tube. Discharge gas was monitored by a flow meter (0–400 mL/min). The plasma was generated inside the tube of LTP probe. Samples were put on a glass slide placed on a 3D moving stage. The distance between the LTP probe tip and analyte surface was 10–25 mm. The analytes were directly desorbed from drug surfaces and ionized by the plasma. The ions were transported to the mass spectrometer. The distance between the LTP probe tip and the vacuum interface of the mass spectrometer was 10–15 mm.

Results and discussion

Optimization of operation parameters

To obtain the highest signal-to-noise ratios of target analytes, several parameters including discharge gas types, flow rates, and discharge powers were optimized. Paracetamol was selected as a representative case by tuning on the protonated molecular ion $[M+H]^+$ at *m*/*z* 152 since this drug is a popular nonsteroidal anti-inflammatory and gives a very simple mass spectrum by the LTP probe at ambient condition.

Effect of gas types and flow rates

Gas types and flow rates are important factors for ionization efficiency by the LTP probe. Helium, nitrogen, and argon are common gases to generate plasmas in DBD ion source [24]. Herein, these three discharge gases were investigated at flow rates of 0–350 mL/min. As illustrated in Fig. 2a, the highest signal intensities are obtained at the flow rate of 300 (an I.D. of 1.0 mm and a gas flow rate of 300 mL/min, resulting in a gas speed of about 6.4 m/s), 150 and 150 mL/min for helium, nitrogen, and argon gases, respectively. The maximum intensity of ion at *m*/*z* 152 is achieved using helium gas at the flow rate of 300 mL/min. In comparison with the gas flow rates in DESI (>350 m/s) [25] and DART (1,500–3,000 mL/min) [14], lower flow rate was required by LTP probe.

Effect of discharge powers

The discharge power has also an influence on the analysis of pharmaceuticals. The relative ion intensities of paracetamol at *m*/*z* 152 are shown in Fig. 2b by using three different powers (5, 18, and 30 W). As shown in Fig. 2b,

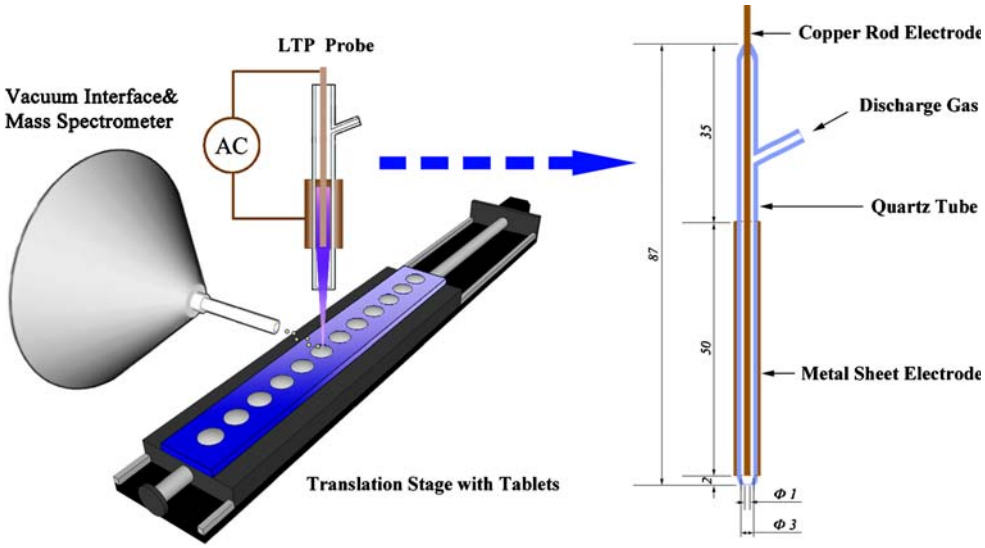

Fig. 1 Schematic diagram of LTP probe for ambient ionization MS (unit, mm)

the signal intensity with discharge gas of helium is always higher than those of argon and nitrogen with same power. For helium gas, the signal intensity increases with the increase of discharge power. The surfaces of drugs showed no damages under discharge power of 5 W. When higher discharge power was used (for example, 30 W), sample surfaces were eroded. Therefore, to avoid damages to the tablets by heating under high discharge power, we used a 5 W power throughout all experiments followed, which generated a plasma with a temperature comparable to surroundings. Helium gas at a flow rate of 300 mL/min was chosen to generate plasma.

Mass spectral characteristics

Antipyretic analgesics drugs

Paracetamol, chlorzoxazone, nimesulide, and meloxicam, which are widely used to relieve pain and diminish inflammation, were investigated. Except for meloxicam, both protonated and deprotonated molecule can be detected for paracetamol, chlorzoxazone, and nimesulide (Fig. 3a–g). In case of paracetamol, abundant peaks of *m*/*z* 152 (Fig. 3a) and *m*/*z* 150 (Fig. 3d) corresponding to protonated and deprotonated molecule are achieved, respectively. The ion of *m*/*z* 303 (Fig. 3a) and *m*/*z* 301 can also be detected (Fig. 3e), which is attributed to $[2M+H]^+$ and $[2M-H]^-$. The results are very similar to previous works of paracetamol by DAPCI, DESI, and DART [26]. The peaks of dimer ions are seen in the spectra. This indicates that occurrence of chemical reactions possibly accompany the ionization process. The ion detected at *m*/*z* 110 are formed by the loss of ketene (42 Da) from parent ions of *m*/*z* 152 (inset in Fig. 3a), which is consistent with the results using DESI [12].

Hormone drugs

Hormone drugs, medroxyprogesterone acetate, dexamethasone acetate, and diethylstilbestrol were analyzed by LTP probe. The acquired full-scan mass and MS/MS spectra are shown in Fig. 4a–d. For diethylstilbestrol, protonated

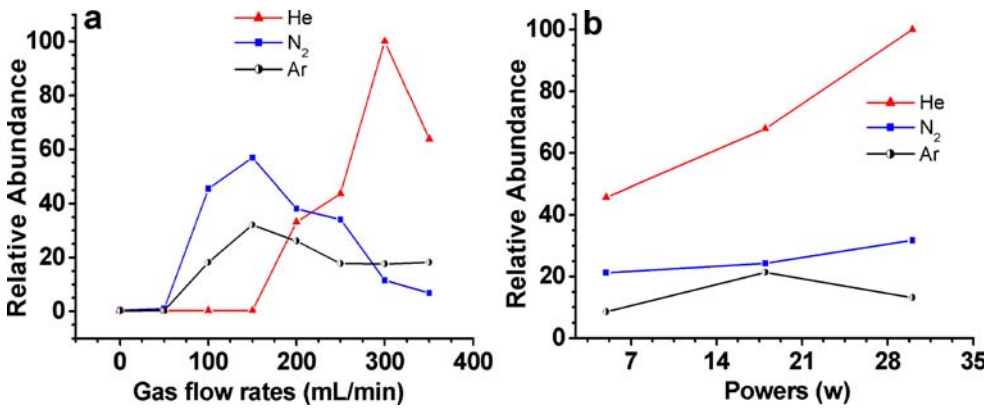

Fig. 2 Optimization of **a** gas flow rates and **b** powers. All the signals were observed based on the ion at *m*/*z* 152

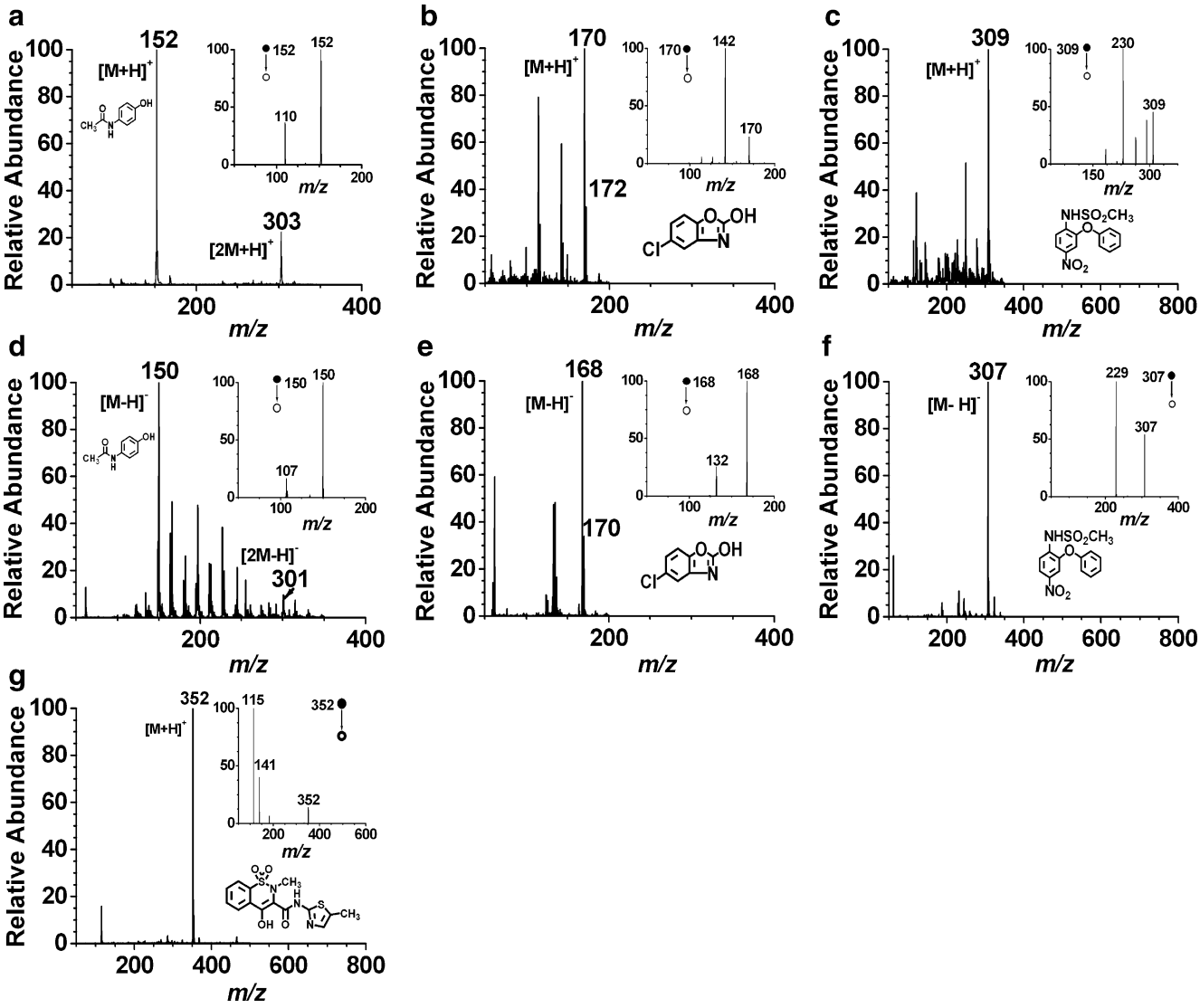

Fig. 3 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in drugs **a** paracetamol, **b** chlorzoxazone, **c** nimesulide, and **g** meloxicam and mass spectra and MS/MS spectra (*inset*) obtained in negative ion mode obtained for **d** paracetamol, **e** chlorzoxazone, and **f** nimesulide granules

molecule at *m/z* 269 (Fig. 4a) and deprotonated molecule detected at *m/z* 267 are observed (Fig. 4c). The signals of active ingredients in tablets, such as medroxyprogesterone acetate at *m/z* 387 (Fig. 4b) and dexamethasone acetate at *m/z* 435 (Fig. 4c), are obtained only in positive ion mode. The fragment ions of *m/z* 327 result from the loss of CH_3CO_2H (60 Da) from the deprotonated molecule (inset in Fig. 4b).

Cardiovascular drugs

The drugs Captopril, nimodipine, flunarizine hydrochloride capsules, and nifedipine are used to treat cardiovascular problems. Mass spectra obtained from these drugs are shown in Fig. 5a–f. In the case of captopril, the peaks of protonated ion at *m/z* 218 and the dimer $[(C_9H_{14}NO_3)2S-S+H]^+$ at *m/z* 433 are observed in positive ion mode, as shown in Fig. 5a, while the ion peak of deprotonated dimer at *m/z* 431 (Fig. 5d) is obtained in negative ion mode, which is due to the formation of disulfide from two captopril molecules. Tandem MS of the deprotonated molecule generates a prominent signal at *m/z* 182 due to a loss of the hydrogen sulfide (inset in Fig. 5d). Both protonated and deprotonated molecule ions of nimodipine are observed in positive and negative ion modes, respectively (Fig. 5b, e). For flunarizine hydrochloride capsules,

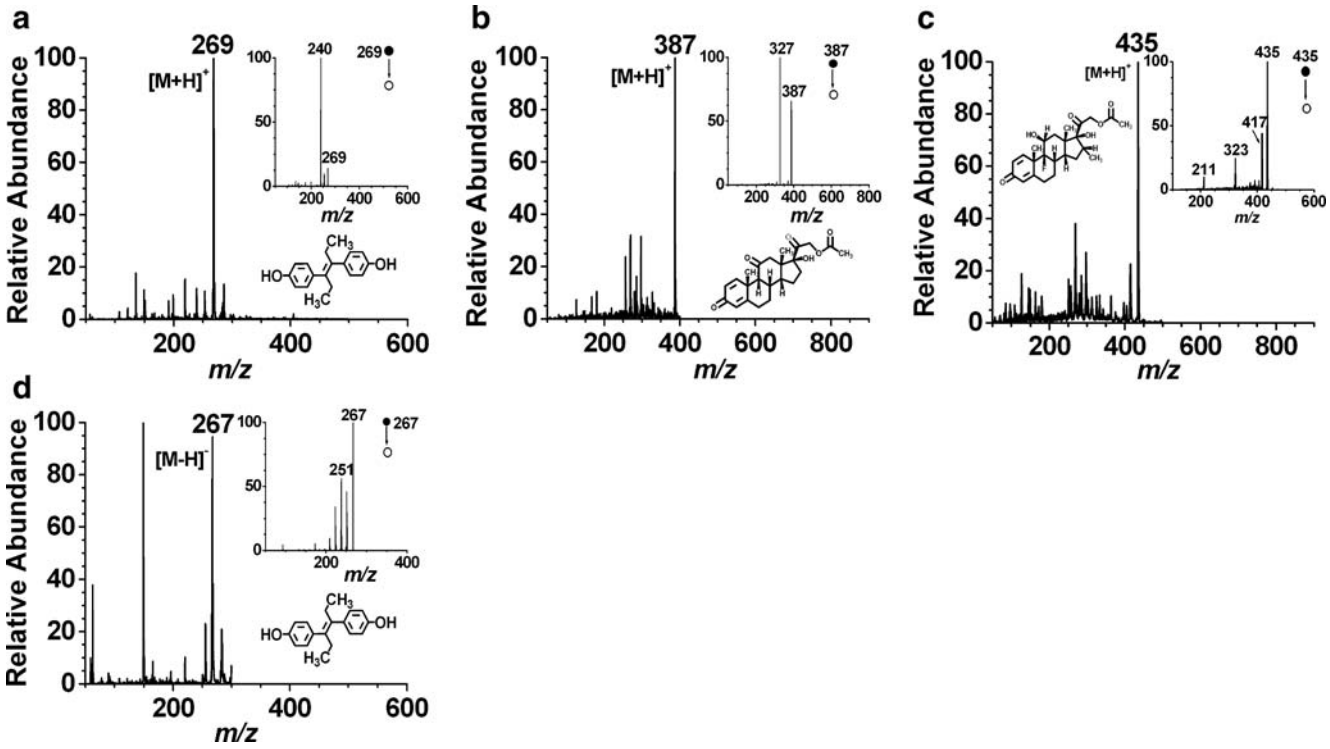

Fig. 4 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in tablets **a** diethylstilbestrol, **b** medroxyprogesterone acetate, and **c** dexamethasone acetate and mass spectrum and MS/MS spectrum (*inset*) obtained in negative ion mode obtained for **d** diethylstilbestrol

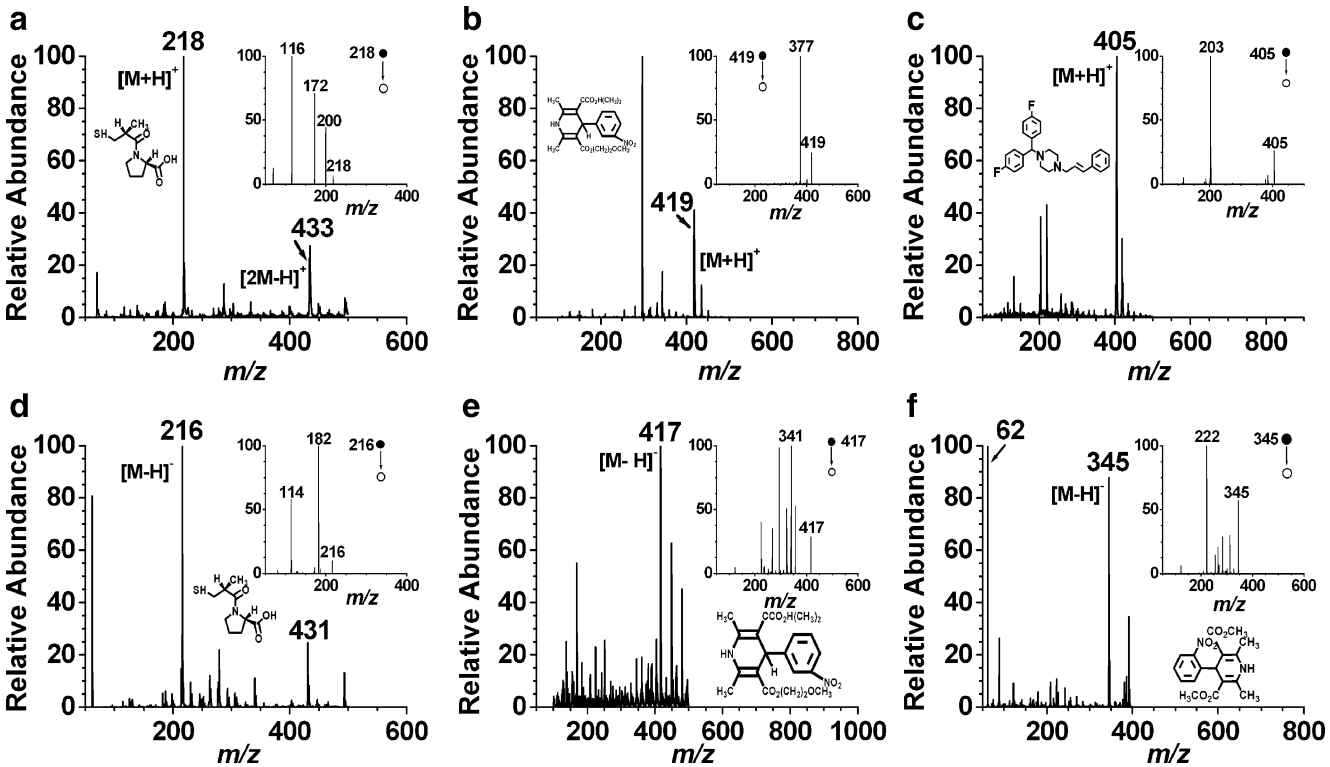

Fig. 5 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in pharmaceuticals **a** captopril, **b** nimodipine, and **c** flunarizine hydrochloride capsules and mass spectra and MS/MS spectra (*inset*) obtained in negative ion mode obtained for **d** captopril, **e** nimodipine,and **f** nifedipine

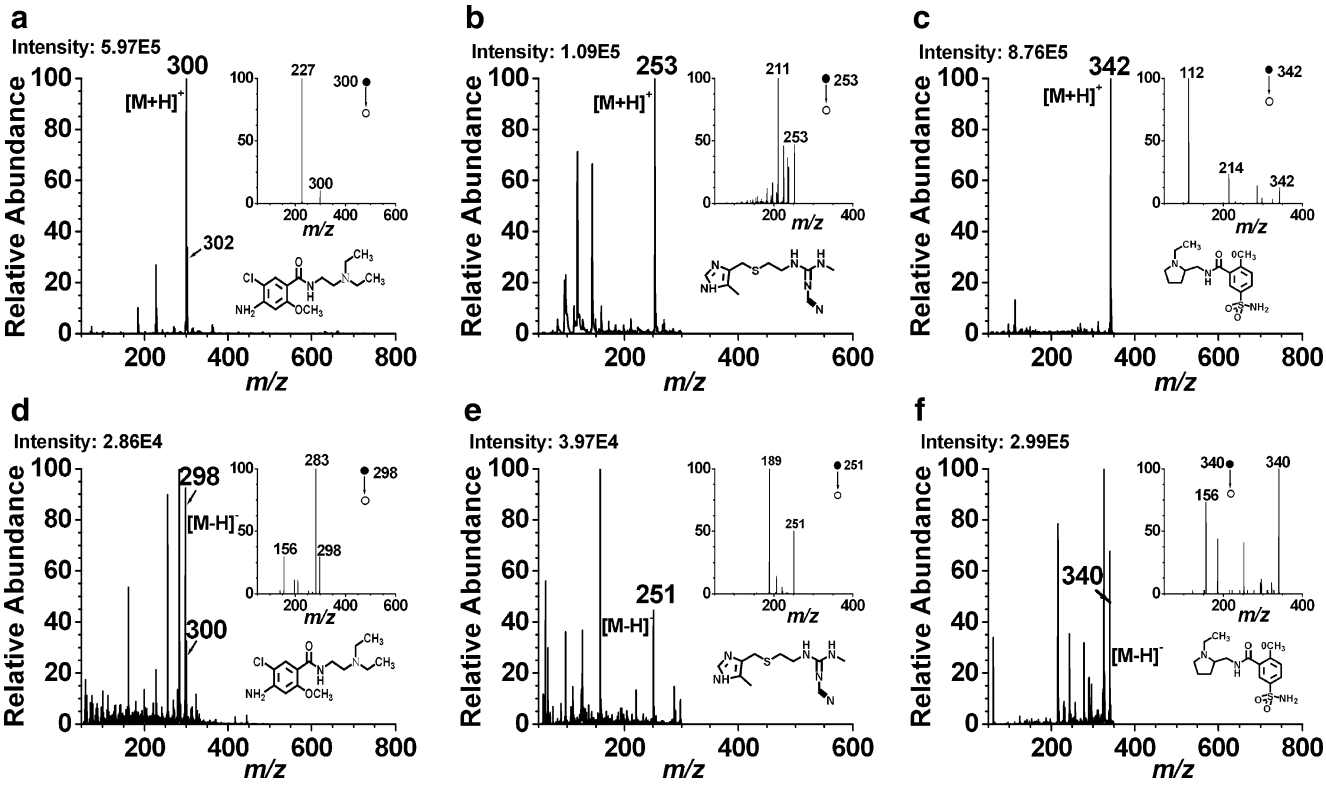

Fig. 6 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in tablets **a** metoclopramide, **b** cimetidine, and **c** sulpiride and mass spectra and MS/MS spectra (*inset*) obtained in negative ion mode obtained for **d** metoclopramide, **e** cimetidine, and **f** sulpiride

the peak of the active ingredient at *m*/*z* 405 is obtained only in positive ion mode in Fig. 5c. On the contrary, the peak at *m*/*z* 345 corresponding to nifedipine is seen only in negative ion mode, as indicated in Fig. 5f.

Digestive system and neuro-psychotherapeutic drugs

Metoclopramide and cimetidine as representative drugs were used to treat digestive system diseases measured. Sulpiride that belongs to neuro-psychotherapeutic drug was also studied. Though they possess different chemical properties of the compounds, the ion peaks of active ingredients can be obtained in both positive and negative ion modes by LTP probe, as shown in Fig. 6a–f. However, signal intensities of all target analytes are higher in positive ion mode than those in negative ion mode. For metoclopramide, the peaks at *m*/*z* 300 and at *m*/*z* 298 are recorded in full-scan mass corresponding to protonated (Fig. 6a) and deprotonated molecules (Fig. 6d), respectively.

Antithyroid and diuretic drugs

Propylthiouracil and thiamazole, which are typical antithyroid drugs, were analyzed. The active ingredients of these two drugs containing thiol functional groups, dimers were either obtained from the reaction of high concentration of monomers in the gas phase or directly desorbed from the surface. For example, protonated dimer $[(C_4H_5N_2)2S_2+H]^+$ formed from two thiamazole monomers appeared at *m*/*z* 227, as shown in Fig. 7b. Collision-induced dissociation of the *m*/*z* 115 peak yields the peak at *m*/*z* 88, a loss of HCN (27 Da; inset in Fig. 7b). The ionization efficiency is influenced by a number of factors, including the chemical properties of analytes (molecular structure, polarity, volatility, etc.) and additives in the tablets. Peaks for additives also appear in the spectra in Fig. 7b. Therefore, the ionization efficiency of thiamazole together with a range of additives in the tablets is comparatively lower due to ion suppression. For furosemide and acetazolamide as representative diuretic drugs, the signals of the active ingredients are obtained only in negative ion mode in Fig. 7c, e, respectively.

Sedative-hypnotics, antiparastic, and sulfa anti-inflammatory drugs

Estazolam as a popular sedative-hypnotics drug was analyzed. The mass spectrum of estazolam tablet by LTP

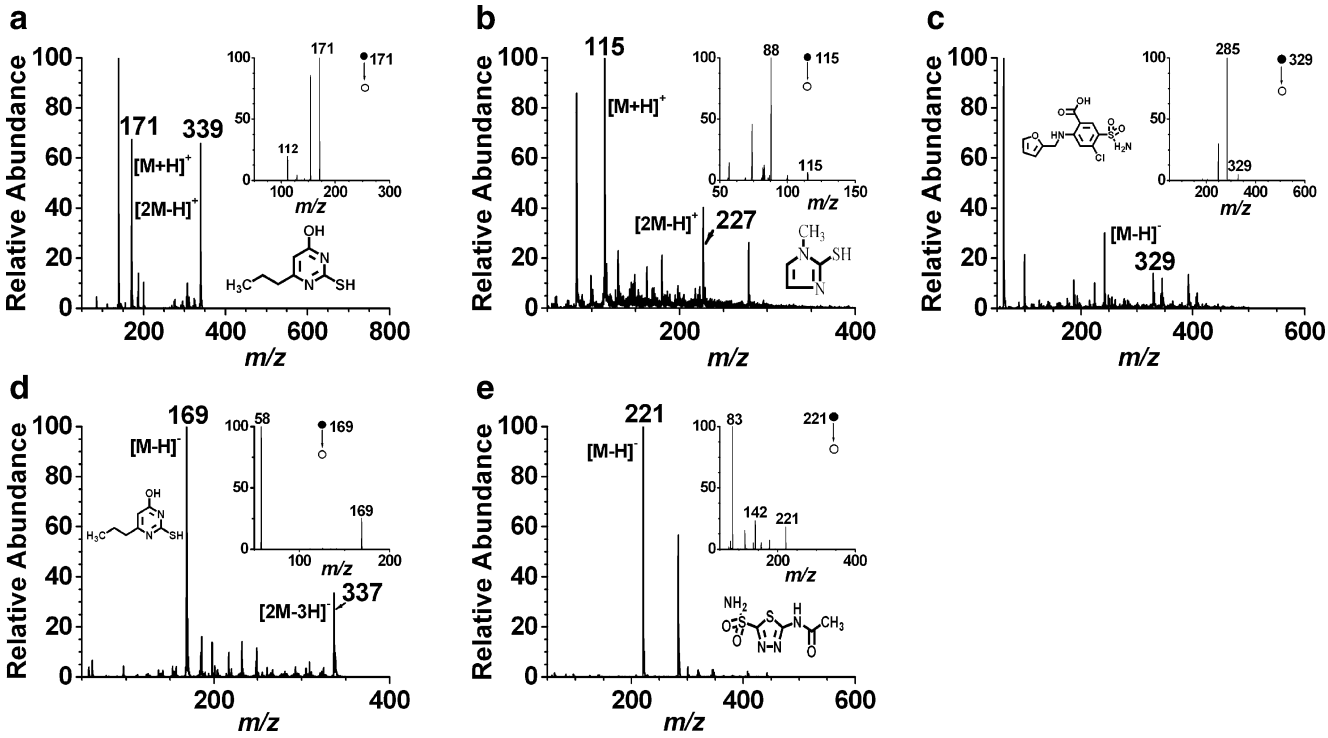

Fig. 7 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in tablets **a** propylthiouracil and **b** thiamazole and mass spectra and MS/MS spectra (*inset*) obtained in negative ion mode obtained for **c** furosemide, **d** propylthiouracil, and **e** acetazolamide

probe is obtained with a characteristic chlorine isotopic signature. As shown in Fig. 8a, the base peak of protonated molecule is observed at *m/z* 295, and ^{37}Cl isotope peak appears at *m/z* 297, with an abundance ratio of 3:1. The dominant fragment at *m/z* 267 in the MS/MS spectrum corresponds to loss of N_2 and is in agreement with results obtained previously [27, 28]. Metronidazole as a representative antiparastic drug was analyzed by MS with LTP probe only in positive ion mode (Fig. 8b), whereas nitrofurantoin tablet as an antibacterial drug was detected only in negative ion mode (Fig. 8c).

Drugs containing more than one active ingredient

For tablets containing more than one active ingredient, expected protonated forms of the active molecules are simultaneous detected. As a representative example, the multiple active ingredients in the aminopyrine phenacetin tablets were determined in positive ion mode. Three active ingredients involve two classifications of pharmaceutical drugs. Aminopyrine and phenacetin are antipyretic analgesics, while caffeine is used as neuro-psychotherapeutic drug. The active ingredients with different properties can be

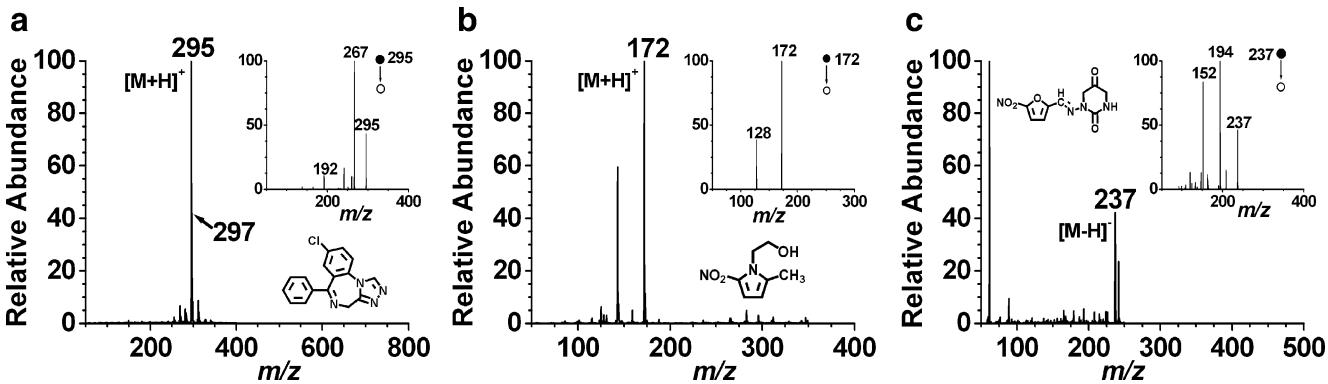

Fig. 8 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in tablets **a** estazolam and **b** metronidazole and mass spectrum and MS/MS spectrum (*inset*) obtained in negative ion mode obtained for **c** nitrofurantoin

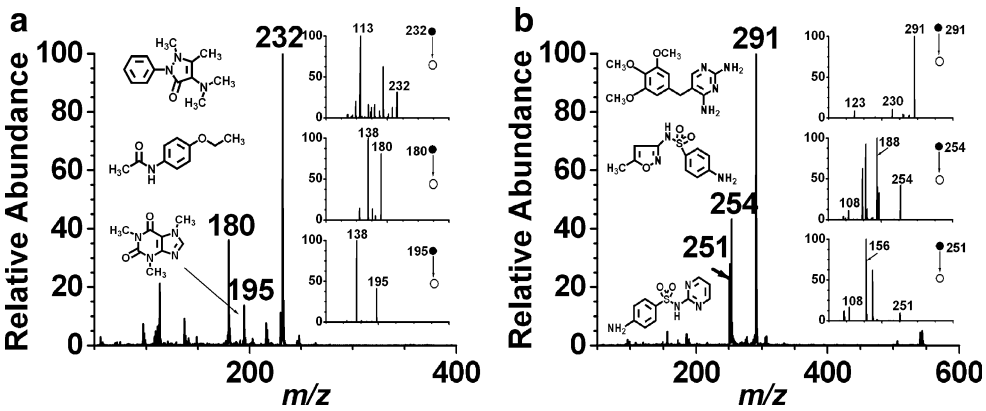

Fig. 9 Mass spectra and MS/MS spectra (*inset*) obtained in positive ion mode by the LTP probe for rapid detection of active ingredients in tablets **a** compounds: aminopyrine, phenacetin, and caffeine and **b** compounds: trimethoprim, sulfamethoxazole, and sulfadiazine

simultaneously analyzed in a single full MS scan (protonated molecule ions aminopyrine at *m/z* 232, phenacetin at *m/z* 180, and caffeine at *m/z* 195), as shown in Fig. 9a. The peaks of their corresponding dominant product ions are seen at *m/z* 113, 138, and 138, respectively. The data confirm that the ion at *m/z* 138 is formed by loss of methyl isocyanate ([M–H_3CN=C=O+H]) from protonated caffeine at *m/z* 195. The MS/MS spectrum is very similar to the results reported previously [26, 29].

Synergic sulphonamide compound tablets consisting of multiple active ingredients belonging to sulfa anti-inflammatory group were studied as another example. A solid tablet containing 80 mg of trimethoprim, 200 mg of sulfamethoxazole, and 200 mg of sulfadiazine were analyzed in positive ion mode by MS using LTP probe. The protonated molecules of each of the active ingredients are shown in Fig. 9b, trimethoprim at *m/z* 291, sulfamethoxazole at *m/z* 254, and sulfadiazine at *m/z* 251. The major peaks from MS^2 spectra are present at *m/z* 230, 188, and 156, respectively, same as described previously [30].

For all the tablets analyzed by LTP probe in both positive and negative ion mode, information including (1) molecular weight, (2) amount of active ingredients in drugs, (3) corresponding forms of molecular ions, and (4) main fragments in the MS/MS spectra are summarized in Electronic supplementary material Table S-1. From this study, it is clear that LTP probe is capable of detection of multiple active components in solid drugs such as tablets using the positive ion detection mode.

High-throughput analyses of drugs

Rapid screening of drug tablets was carried out by choosing methyltestonterone as a representative. The intensity of protonated molecule at *m/z* 303 is relatively high in the positive ion mode as shown in Fig. 10a. It took 3 s to analyze one tablet. However, the problem of low resolution appeared in total ion current (TIC) or extracted ion current (EIC) when the interval between two tablets analyzed was less than 3 s. Memory effect and the response time of mass spectrometer probably result in the low resolution. To eliminate memory effect and offer more scanning time for the MS to give a relatively good mass spectrum, a time interval of 3 s is selected. Therefore, it needs approximately 6 s in total to analyzed one single tablet. TIC for 18

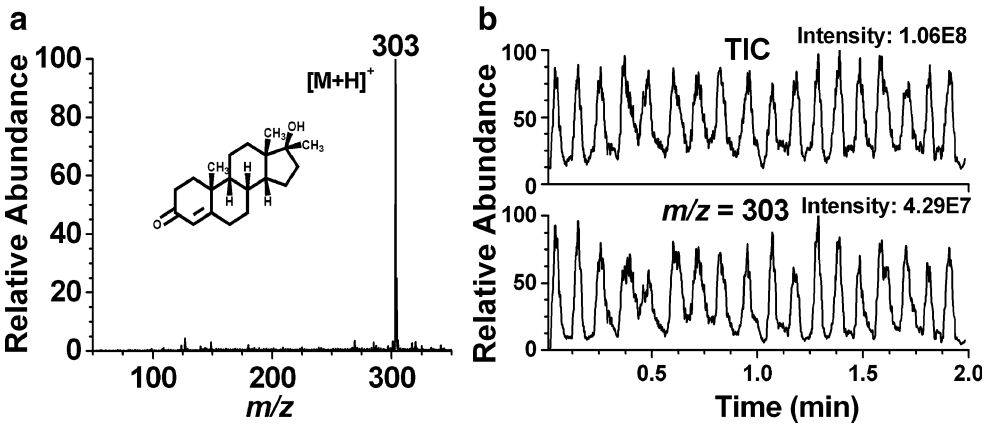

Fig. 10 Mass spectrum of methyltestonterone (**a**), TIC and EIC (*m/z* 303) of 18 sequential analysis of methyltestonterone tablets (**b**). The experiments were performed in positive ion mode by the LTP probe, and mass spectra were smoothed using a 15-point boxcar filter

methyltestonterone tablets is illustrated in Fig. 10b. Eighteen identical tablets were scanned within 1.9 min. The relative standard deviation of the integrative peaks in EIC is 18.5%. These results demonstrate the feasibility of utilizing the LTP probe for the effective ionization as a rapid qualitative screen of the active ingredients in drugs.

Conclusions

In summary, the results above demonstrated that a LTP probe is a potential ion source for rapid qualitative screening of a wide variety range of active pharmaceutical ingredients in various structure compounds at ambient conditions. The feasibility of utilizing the LTP probe for rapid screening was explored by analyzing 18 identical pharmaceutical tablets within 1.9 min. An experimental platform allows 600 samples to be detected in 1 h, which is a considerably high-throughput technique. The LTP probe offers the following advantages: (1) it is convenient to detect pharmaceuticals in both positive and negative ion modes without changing the polarity of high voltage power. The analytes can be detected in either positive or negative mode by switching the positive/negative button of the software to allow analyses of either positive or negative ions in the plasma; (2) no sample preparation or pretreatment is needed in pharmaceutical analysis; (3) pharmaceuticals were not contaminated because no solvents were used in the experiments; (4) the developed technique could be potentially applied for high-throughput analysis, providing a method for drug quality monitoring in a real-time approach.

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