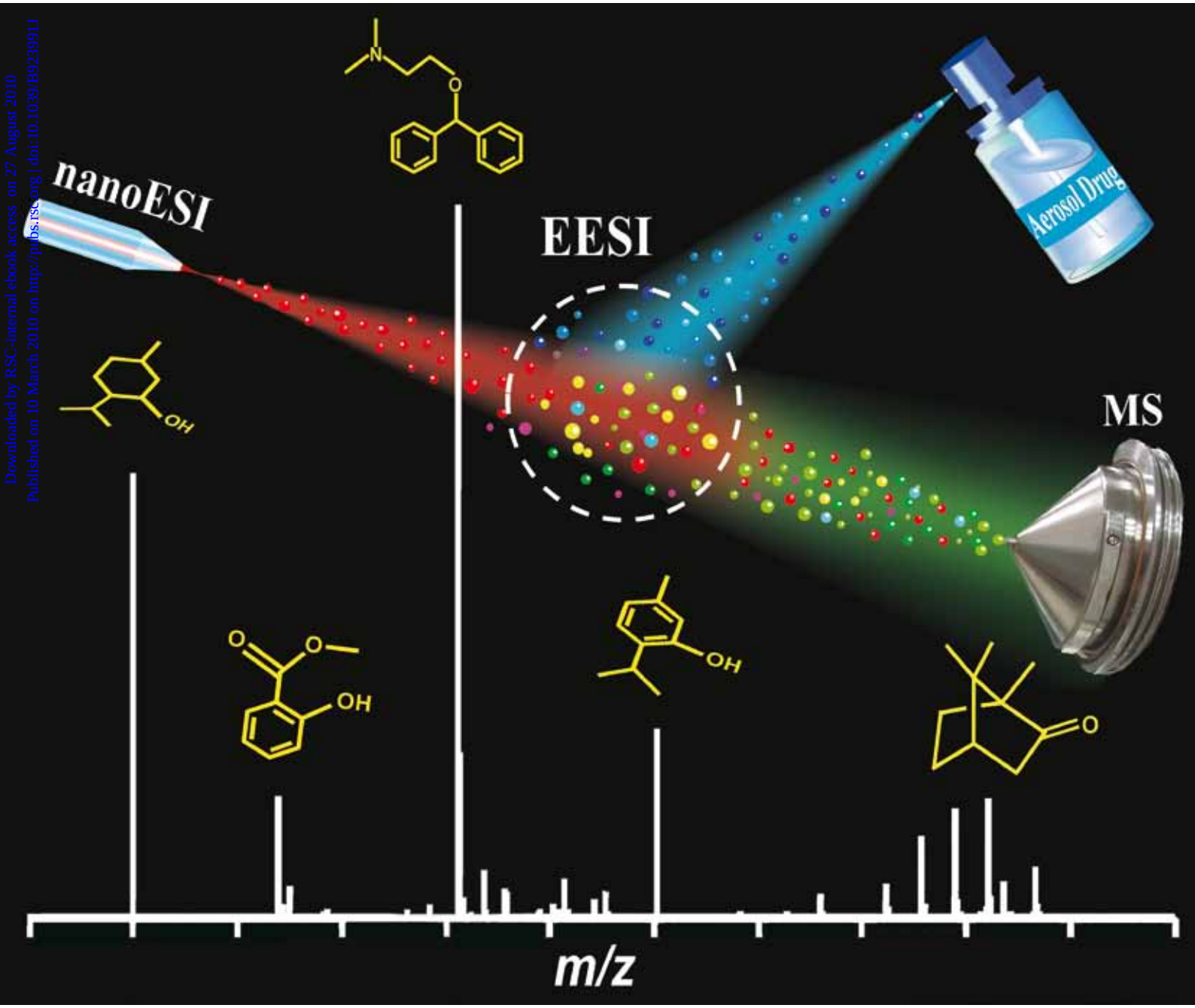
Analyst

Interdisciplinary detection science

www.rsc.org/analyst

Volume 135 | Number 6 | June 2010 | Pages 1157–1464



ISSN 0003-2654

RSC Publishing

CRITICAL REVIEW
Laurel E. Averett and Mark H. Schoenfisch
Atomic force microscope studies of fibrinogen adsorption

PAPER
Huanwen Chen *et al.*
Rapid analysis of aerosol drugs using nano extractive electrospray ionization tandem mass spectrometry

0003-2654(2010)135:6;1-J

Rapid analysis of aerosol drugs using nano extractive electrospray ionization tandem mass spectrometry†

Haiwei Gu, Bin Hu, Jianqiang Li, Shuiping Yang, Jing Han and Huanwen Chen*

Received 13th November 2009, Accepted 19th February 2010
First published as an Advance Article on the web 10th March 2010
DOI: 10.1039/b923991j

Aerosol drugs dominate a significant share of pharmaceutical preparations on the market. A novel sensitive method utilizing nano extractive electrospray ionization mass spectrometry (nanoEESI-MS) has been developed for the rapid analysis of aerosol drug samples with quantitative information. Without any sample pretreatment, aerosol drugs were manually sprayed into the primary ion plume created by a nano electrospray emitter for direct ionization under ambient conditions. The analyte ions of interest were guided into an ion trap mass spectrometer for tandem mass analysis. The active ingredients of various aerosol drugs, such as econazole nitrate, beclomethasone dipropionate, binary mixture of methyl salicylate and diphenhydramine, terbutaline, and salbutamol, were rapidly detected using nanoEESI-MS. A single sample analysis could be completed within 1.2 s. Tandem mass spectrometry was used to confirm the identification of important compounds in each aerosol drug sample. Reasonable relative standard deviation (RSD = 6.39%, $n = 13$) and acceptable sensitivity (10 ppt, 100 μL) were found for the salbutamol aerosol sample, which suggests that nanoEESI-MS has the quantitative capacity for analyzing complex pharmaceutical samples. This method was further extended to study the thermal decomposition process of salbutamol, showing that the degradation kinetics of salbutamol can be conveniently tracked. Our data demonstrate that nanoEESI tandem mass spectrometry is a fast and sensitive technique for the analysis of aerosol drug preparations, showing promising applications in pharmacology studies and *in situ* analysis of aerosol drugs on the market.

Introduction

The rapid and sensitive detection of active ingredients and impurities in pharmaceuticals is one of the most challenging topics in modern analytical chemistry and pharmacology studies.[1–5] Aerosol drugs, also known as inhalation therapy or nebulized drug therapy, are typical over-the-counter drugs which have played irreplaceable roles in treating skin problems and lung/airway diseases[6] including asthma, tinea, sore pain, and bronchial airway diseases. Aerosol drugs are delivered into the patent's body in the form of tiny droplets, often bound to liquid/gaseous substances, *via* air using an inhaler. Thus, aerosol drugs usually have high pressure and complex matrices, which makes it difficult to directly determine the active compounds. The active components in drug preparations are usually sensitive to light and/or heat. The quality of drug may degrade significantly during the transportation and storage, as well as from improper usage. Expired drugs lose efficacy and may become toxic; therefore it is very dangerous for patients to take expired drugs. Protecting people from ineffective or dangerous drugs on the market and accelerating the pharmacological studies of drugs require novel analytical techniques for the fast, sensitive, and specific detection of trace amounts of targeted compounds such as active ingredients present in the complex aerosol drug matrices. In industry, quality control of aerosol drugs is carried out on the raw materials, during production, and on the finished products. Aerosol drug ingredients are tested by the very similar methods as those for non-aerosols; aerosol delivery devices are characterized by measuring discharge rate, leakage, dosage, particle size, and *etc.*[7–9] Important analytical techniques include gas chromatography (GC), liquid chromatography (LC), Raman spectroscopy, atomic force microscopy (AFM), and mass spectrometry (MS).[10–16] Complex and laborious sample pretreatments are frequently required in the routine methods currently available, while reports of fast and *in situ* analysis on aerosol drug products are seldom seen.[17]

Due to its high sensitivity and specificity, mass spectrometry has been widely used in the analysis of complex mixtures including wine, tea, biofluids, plant extractions, egg samples, drugs, and *etc.*[5,18–24] Conventional mass spectrometry-based methods such as liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) require multiple-step sample pretreatments such as sample degassing, extraction, and derivatization before sample analysis.[2,25] The sample pretreatment process is usually time consuming, and thus makes the high throughput analysis of pharmaceutical preparations impossible. The best solution available to the bottleneck of rapid mass spectrometric analysis is ambient mass spectrometry, because it requires minimal sample pretreatment and enables high throughput analysis of actual samples without serious sensitivity loss.[26] Numerous applications

College of Chemistry, Biology and Material Science, East China Institute of Technology, Fuzhou, Jiangxi Province, 344000, P. R. China. E-mail: chw8868@gmail.com; Fax: (+86)-794-8258-320

† Electronic supplementary information (ESI) available: Diagram of the proposed dissociation mechanism of econazole and tandem mass spectra for the identification of diphenhydramine, thymol, camphor, methyl salicylate, and menthol. See DOI: 10.1039/b923991j

have been demonstrated using ambient ionization techniques including desorption electrospray ionization (DESI),[27–31] direct analysis in real time (DART),[32–35] extractive electrospray ionization (EESI),[36–38] desorption atmospheric pressure chemical ionization (DAPCI),[20,39,40] desorption atmospheric pressure photon ionization (DAPPI),[41] dielectric barrier discharge ionization (DBDI),[42] low-temperature plasma (LTP),[43–45] and many combinations of desorption with post-ionization methods working at atmospheric pressure (for a review, see ref.46). For the detection and identification of interesting compounds in aerosol samples, DESI-MS has been used for analyzing dried aerosol particles which were generated using a fluidized bed powder disperser and then directed toward the inlet of mass spectrometer.[47,48] Satisfactory results were obtained in all the reported studies, where most efforts were made to detect major components in solid phase. In contrast, a method based on ambient mass spectrometry for the direct detection of aerosol drugs in their original matrices has rarely been seen in previous literature.

Obviously, it is of great importance to develop a sensitive and *in situ* analysis technique to quickly screen impurities and/or degradation products of aerosol drugs on the market. Due to its unique design (Fig. 1), EESI is an effective ionization source for carrying out this challenging task under ambient conditions. EESI utilizes two sprays and aligns them along a certain angle with respect to the mass spectrometer. Neutral samples from one spray can be ionized by primarily charged droplets generated by the electrospray spray. Advantageously, EESI-MS allows the direct analysis of samples in either gas or condensed phase. During EESI analysis, neutral samples and charged solvent droplets are continuously introduced to the spatial cross section in front of the MS inlet, such that the neutral analytes are favorably ionized under the ambient conditions. The matrices of samples are dispersed in a relatively large space between the neutral sample spray, the electrospray, and the ion entrance of the instrument. This enables EESI to tolerate extremely complex matrices, and thus a promising technique for directly analyzing pharmaceutical samples such as aerosol drug preparations. Another merit of EESI is that samples are well isolated from the direct bombardment by charged particles or energetic metastable atoms, and thus it is a relatively soft ionization method. EESI has been applied to the direct characterization of perfumes,[49,50] metabolic biomarkers in biological samples,[51–53] trace amounts of explosives on human skin,[54] and drugs[17] without sample pretreatment, even in a remote distance.[53–56]

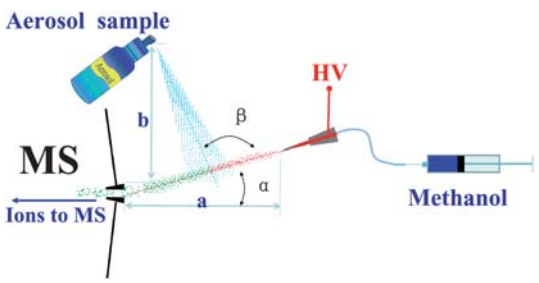


Fig. 1 Schematic diagram of the nanoEESI setup for rapid aerosol drug analysis.

In the present study, we used a novel approach based on a nanoEESI source implemented on a commercial LTQ-XL mass spectrometer to directly analyze five aerosol drug preparations including salbutamol aerosol, terbutaline aerosol, econazole nitrate aerosol, binary mixture of methyl salicylate and diphenhydramine aerosol, and beclomethasone dipropionate nasal aerosol. These drugs were chosen because they are widely used and they are typical representatives of different complexity levels. Since nanoEESI-MS requires no sample pretreatment, rapid analysis was successfully performed on aerosol samples. The time to analyze a typical sample was 1.2 s. Tandem mass spectrometry was applied to confirm the detection of active ingredients in individual aerosol drugs. Acceptable RSD values and sensitivity show that nanoEESI-MS is a promising tool for the quantitative analysis of aerosol drugs. Furthermore, using salbutamol as an example, nanoEESI-MS was able to track the degradation kinetics of active ingredients in pharmaceutical preparations, which shows its great potential and practicality for use in drug testing and development.

Experimental

Reagents and materials

Methanol (A.R. grade), ethanol (A.R. grade), and camphor were bought from Chinese Chemical Reagent Co. Ltd. (Shanghai, China); thymol (A.R. grade) and methyl salicylate (A.R. grade) were purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China); menthol was purchased from Tokyo Chemical Industry Co. LTD. (Tokyo, Japan). The chemical reagents were directly used without further treatment. Deionized water, provided by the chemistry facility at ECIT, was used for all the experiments.

Five types of aerosol drug preparations were tested in this study. Econazole nitrate spray (Xiuzheng Pharmaceutical Group Co. Ltd., Tonghua, P. R. China), beclomethasone dipropionate nasal aerosol (Glaxo Operations China Limited, Tianjin, P. R. China), binary mixture of methyl salicylate and diphenhydramine spray (Heilongjiang Tianlong Pharmaceutical Co. Ltd., Harbin, P. R. China), terbutaline aerosol (AstraZeneca Pharmaceutical Co. Ltd., Wuxi, P. R. China), and salbutamol aerosol (Shanghai Pharmaceutical Group Co. Ltd., Shanghai, P. R. China) were bought in local drug stores. All the aerosol drug samples were quality-passed commercial medicine products, and used directly without further treatment, unless indicated specifically.

Econazole nitrate spray. Econazole nitrate is commonly used as an antifungal medication. The commercial spray product contains econazole nitrate at a concentration of 0.01 g mL^{-1} and other ingredients such as propanediol, tween 80, and ethanol (major matrix, > 99.6%).

Beclomethasone dipropionate nasal aerosol. Beclomethasone dipropionate nasal aerosol is widely used for the relief of symptoms of allergic rhinitis. The commercial product is composed of a large amount of ethanol and a low concentration (0.154%, w/w) of beclomethasone dipropionate. According to the product specification, the total amount of beclomethasone dipropionate is 50 μg for each actuation.

Binary mixture of methyl salicylate and diphenhydramine spray. Binary mixture of methyl salicylate and diphenhydramine spray is designed with multiple active components for arthralgia, muscle pain, low back pain, and pain caused by bruises and tennis elbow. For the commercial product (50 mL bottle), the active compounds include methyl salicylate (1.5 g), diphenhydramine hydrochloride (0.09 g), menthol (1.2 g), camphor (1.95 g), and thymol (0.15 g). For each actuation, a 0.167 mL portion of the solution (defined by the manufacturer) is dispensed.

Terbutaline sulfate aerosol. Terbutaline sulfate aerosol is an effective bronchodilator agent used for patients with reversible obstruction in the airways. The commercial product is a diluted terbutaline sulfate ethanol solution. Each actuation sprays 0.25 mg terbutaline sulfate.

Salbutamol aerosol. Salbutamol is also a bronchodilator to make breathing easier, which works by relaxing the muscles and air passages of the lungs. The commercial product is a diluted salbutamol ethanol solution with a low concentration of 0.2% (w/w). Each actuation contains 0.14 mg salbutamol.

To study the thermal decomposition process of salbutamol, 5 mL water was added into a drug container which contained a 3.8 mL sample of salbutamol (commercial aerosol drug product) to make a solution of 8.8 mL. This solution was heated at 93 °C for more than 4 h using a water bath. Note that this container sprays 0.1 mL for each actuation. After a regular time interval (*e.g.*, 1 h), the drug container was rapidly cooled down to the room temperature using the cool tap water (22 °C); then 0.1 mL salbutamol sulfate was manually sprayed into the nanoEESI source for ionization. The signals of salbutamol and its degradation products (*m*/*z* 86) were recorded rapidly, and averaged with 5 measurements to plot the thermal decomposition kinetics.

NanoEESI-MS instrument

All the experiments were carried out using a homemade nanoEESI source coupled to a Thermo Finnigan LTQ-XL mass spectrometer (San Jose, CA). The principle and structure of nanoEESI source is shown in Fig. 1. The nanoEESI is mainly composed of a gasless nano electrospray emitter (FS capillary, ID 50 μm, OD 250 μm) for generating the primary ions. The neutral aerosol sample was manually introduced by releasing the aerosol drug stored in the drug container. The nanoEESI source allows the experiment to be performed without sheath gas or discharge gas, eliminating the need for a cumbersome gas cylinder. This makes the nanoEESI source attractive for direct *in situ* analysis of aerosol drug samples. The nano electrospray emitter was placed 4 cm (a) away from the inlet of the mass spectrometer. The tip of the aerosol emitter was set 6 cm (b) away from the ion entrance of the mass spectrometer. The nanoEESI source was coaxially mounted to the mass spectrometer, and the aerosol emitter was appropriately fixed onto the mass spectrometer so that the aerosol sample was reproducibly introduced to the nanoEESI source. The angle (α) formed between the nanospray emitter and the inlet of the mass spectrometer was 30°; the angle (β) formed between the nano electrospray emitter and the aerosol sample injector nozzle was set to 135°. This configuration helps to avoid sample contamination by spraying the sample in the opposite direction of the inlet of mass spectrometer. A high voltage of +3.5∼5 kV was supplied to the nanoEESI emitter, ensuring that the nanoEESI emitter constantly generates primary ions with high efficiency. The primary ions were created by electrospraying a methanol–water solution at 0.1 μL min^{-1}. The aerosol (Fig. 1) was nebulized toward the charged plume by pressing the trigger of aerosol drug container for about 2∼3 s. The actuation each time was performed in the same way, regarding the position, direction, pushing time, and pushing force (the full strength). The total amount (100∼167 μL) of the material was nebulized toward the charged plume for each actuation, depending on the sample containers used. After each actuation, the sample aerosol lasted about 1.2 s, which was detected through the sample chromatographic peak in the total ion current (TIC) chromatogram recorded using the nanoEESI source. Accordingly, the mass spectra were collected using an average time of 1.2 s.

The analytes in the aerosol samples with complex matrices were directly ionized when the neutral sample plume encountered the primary ion beam generated by the nanoESI emitter; the analyte ions were then introduced to the mass spectrometer under the electrical force and the vacuum of the LTQ instrument. The temperature of the inlet capillary was maintained at 180 °C during analysis. The ion injection time was set to 100 ms for the LTQ-MS. To perform collision-induced dissociation (CID) experiments, ions of interest were isolated using an isolation *m*/*z* window width of 1 unit unless otherwise specified. The collision energy for CID experiments was 10∼30% artificial units with a duration time of 30 ms. The other parameters used in the experiments were default values of the mass spectrometer. All the mass spectra were obtained using the Xcalibur software (with background subtraction).

Results and discussion

Characterization of nanoEESI-MS

The performance of nanoEESI source was optimized using aerosol drug samples including terbutaline sulfate and salbutamol. Terbutaline, a selective β_2-receptor agonist, can be directly sprayed into the throat for the treatment of bronchial asthma, asthmatic bronchitis, emphysema and so on. However, its bronchodilator activity is weaker than salbutamol, but it is much more expensive than the latter. These two drugs were selected as the samples for the nanoEESI characterization, because their importance in clinical therapy and the availability of their nanoEESI data.[17] Fig. 2a shows the nanoEESI-MS spectrum recorded from the terbutaline sulfate aerosol drug sample. The signal at *m*/*z* 226 was attributed to the protonated terbutaline. Upon CID (the inset of Fig. 2a), the precursor ions of *m*/*z* 226 generated product ions of *m*/*z* 208, 170, and 152 by the loss of H_2O, $CH_2{=}C(CH_3)_2$, and [H_2O, $CH_2{=}C(CH_3)_2$], respectively. These observations are in good agreement with the previously reported data,[17] showing that the nanoEESI source worked successfully for terbutaline sulfate aerosol drug detection.

The operating parameters of the nanoEESI were optimized to produce the highest abundance of *m*/*z* 226 using the terbutaline

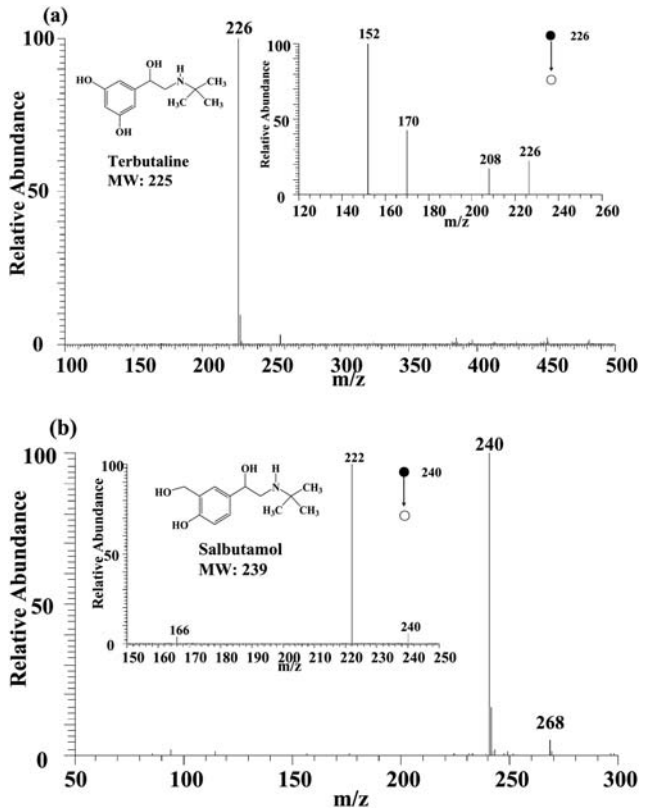


Fig. 2 Mass spectra recorded using the terbutaline sulfate aerosol drug preparation and salbutamol aerosol drug preparation. a): NanoEESI-MS spectrum of terbutaline sulfate solution; the inset shows the MS/MS spectrum of protonated terbutaline (*m/z* 226); b) NanoEESI-MS spectrum of salbutamol aerosol; the inset shows the MS/MS spectrum of protonated salbutamol (*m/z* 240).

sulfate samples. As the result, the distance (b) between the nozzle of sample container and the inlet of the mass spectrometer was optimized to be 6 cm; the distance (a) from the inlet of the mass spectrometer to the tip of the nanoESI emitter was 4 cm; the angle (α) formed between the nanospray emitter and the inlet of the mass spectrometer was 30°; the angle (β) of the nanospray emitter with respect to the nozzle of the sample container was 135°; the electrospray voltage was +3.5∼5 kV; the electrospray solution (methanol–water, 1 : 1) was infused at 0.1 μL min^{-1}. Under the optimized conditions, salbutamol aerosol was also successfully analyzed without any sample pretreatment. As shown in Fig. 2b, the signal at *m/z* 240 corresponded to the protonated salbutamol and the structure was confirmed by the CID experiment (the inset of Fig. 2b). Similar to terbutaline, the precursor ions at *m/z* 240 generated product ions of *m/z* 222, 166 by the loss of H_2O and $CH_2{=}C(CH_3)_2$, successively. These data are in good agreement with previous studies,[17] and thus confirm that under the experimental conditions the nanoEESI source functions properly for rapid aerosol drug preparation analysis.

Analysis of commercial aerosol drugs

Econazole nitrate spray. Aerosol is a collection of small particles and/or droplets in air. The direct and rapid analysis of aerosol drugs has become one of the most interesting topics in aerosol analytical science. Active components of aerosol drugs are delivered in the form of tiny droplets bound to solvent (*e.g.*, ethanol, water, *etc.*) and gaseous substance (*e.g.*, oxygen, nitrogen, *etc.*). Thus, aerosol drugs are a typical type of pharmaceutical samples with complex matrices, challenging many techniques for high throughput analysis. As the first demonstration, nanoEESI-MS was used to analyze econazole nitrate spray. Econazole (average molecule weight: 381.7, containing three chlorine atoms) is the major functional compound. The mass spectrum of econazole nitrate is shown in Fig. 3a, in which the predominant peaks are protonated econazole at *m/z* 381 (with three ^{35}Cl atoms), *m/z* 383 (with two ^{35}Cl atoms and one ^{37}Cl atom) and *m/z* 385 (with two ^{37}Cl atoms and one ^{35}Cl atom). The abundance ratio for these 3 peaks was 3 : 3 : 1, which is in accordance with its natural isotope ratio. Besides the isotope ratio, tandem experiments were applied to the precursor ions of interest, with 10∼30% collision energy to confirm these peak assignments. Being isolated with an *m/z* window width of 1 unit, the precursor ions of *m/z* 383 generated product ions of *m/z* 193 and 195 by the loss of 2,4-dichloroacetophenone (Fig. 3b). While more detailed studies are necessary to confirm the mechanism, it is inferred from the MS/MS spectrum that the fragmentation is probably caused by charge-induced dissociation and rearrangement. As shown in Fig. S1,† the charge might be transferred from N to C and the first dissociation happened at position 1, which caused rearrangement and further dissociation at position 2. The fragments of *m/z* 125 and 127 were ascribed to p-chlorotoluene cations, which were generated by the loss of imidazole from the precursor ions of *m/z* 193 and 195, respectively. Similarly, as shown in Fig. 3c, the peak at *m/z* 381 produced fragments of *m/z* 193 and 125 by the loss of 2,4-dichloroacetophenone and imidazole, successively. These fragments provide solid evidences for the successful detection of econazole in the aerosol sample. The peak at *m/z* 761 was interpreted as the proton bound dimer of econazole molecules, and the adjacent peak (*e.g.*, *m/z* 763) was due to the chlorine isotopes (8 MS scans, Fig. 3a). Upon CID with a 3 unit isolation width, the peak at *m/z* 761 generated abundant isotope peaks at *m/z* 381 and 382 (Fig. 3d), which further cleaved 2,4-dichloroacetophenone and imidazole, successively, in MS^3 experiments to produce ions of *m/z* 193 and *m/z* 125 (inset of Fig. 3d). These data confirmed that the peak at *m/z* 761 was the protonated dimer of econazole, which was formed due to the high concentration in the aerosol drug sample. To be noted, the isotopic ratio in tandem mass spectrometry is strongly affected by the scan number and the isolation width of parent ions using our instrument.

Beclomethasone dipropionate nasal aerosol. Beclomethasone dipropionate (BDP) (average molecule weight: 521, containing one chlorine atom) nasal aerosol was also directly analyzed using the nanoEESI-MS technique without sample pretreatment. In the nanoEESI-MS spectrum (Fig. 4a), the peak at *m/z* 521 (chlorine isotope was ^{35}Cl) was assigned to the protonated BDP; the adjacent peak at *m/z* 523 was due to the chlorine isotope ^{37}Cl. Note that the peak abundance ratio (*m/z* 521/523) was close to 3 : 1, showing the natural isotopic abundance of BDP. In the CID experiments, the precursor ions of *m/z* 521 yielded fragments of *m/z* 503, 485, 411, and 319 by the subsequent loss of

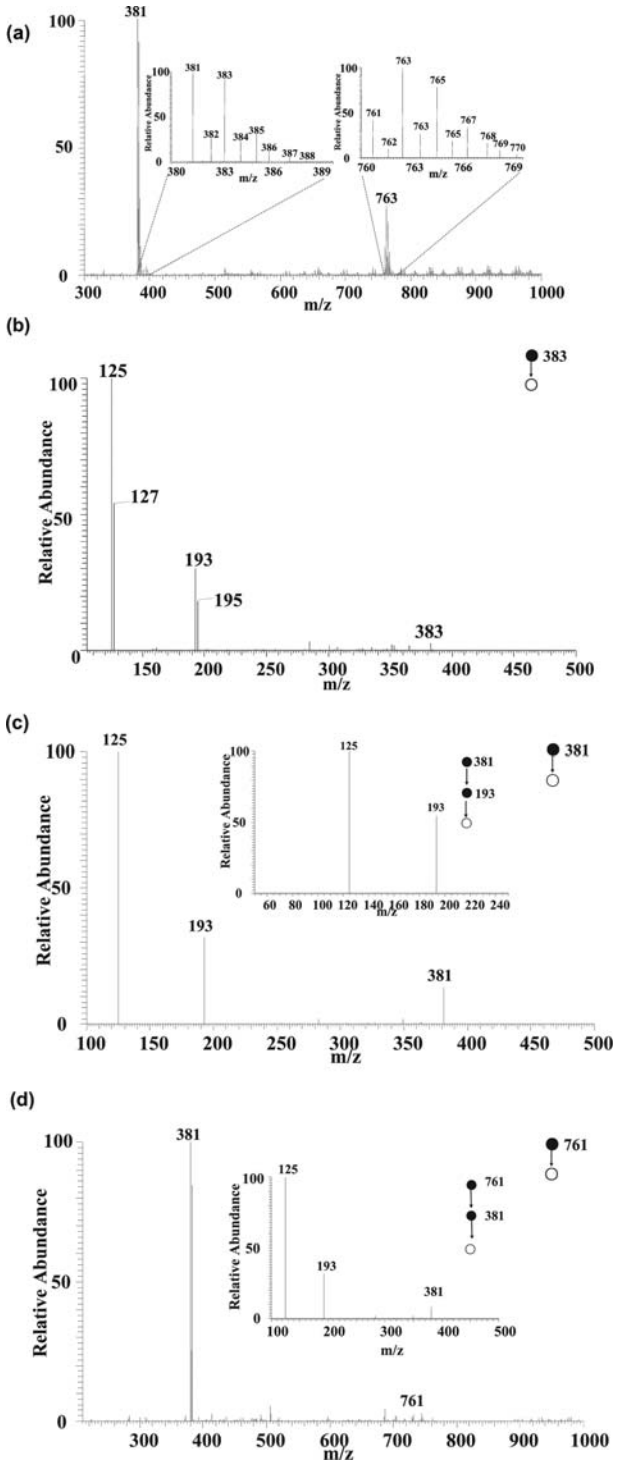


Fig. 3 Rapid nanoEESI mass spectrometric analysis of econazole nitrate aerosol drug products. a): NanoEESI-MS spectrum of econazole nitrate spray sample (8 scans); the insets are amplified view of the *m/z* range 380–390 and 760–770; b): MS/MS spectrum of protonated econazole (*m/z* 383) using a *m/z* isolation width of 1 units; c): MS/MS spectrum of protonated econazole (*m/z* 381) using a *m/z* isolation width of 1 unit, the inset shows the MS^3 spectrum of the product ions at *m/z* 193; d): MS/MS spectrum of proton bound econazole dimmer with an isolation width of 3 units. The inset shows the MS^3 spectrum of the product ions at *m/z* 381.

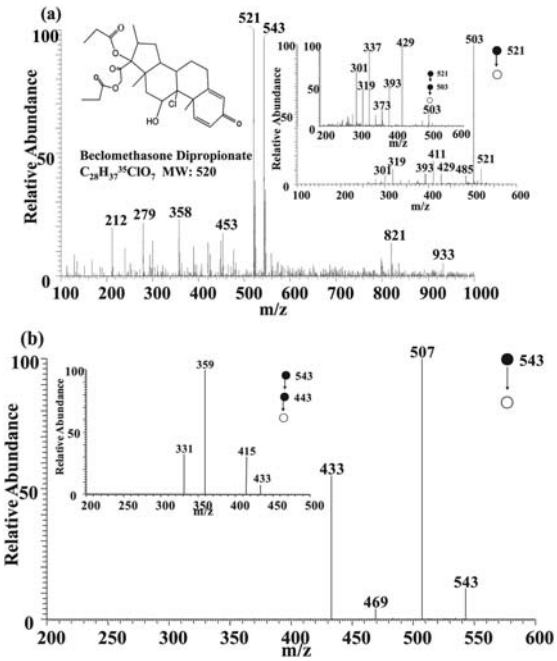


Fig. 4 Rapid nanoEESI mass spectrometric analysis of commercial beclomethasone dipropionate (BDP) nasal aerosol products. a): Nano-EESI mass spectrum of BDP nasal aerosol; the insets show the MS^2 and MS^3 spectra of protonated BDP (*m/z* 521); b): MS/MS spectrum of sodiated BDP; the inset shows the MS^3 spectrum of *m/z* 433.

water, water, propionic acid, and [propionic acid + water] (the inset of Fig. 4a). In the MS^3 spectrum (the inset of Fig. 4a), the precursor ions (*m/z* 503) produced the major fragments of *m/z* 429, 393 by the loss of propionic acid, [hydrogen chloride + propionic acid], respectively. The peak at *m/z* 543 detected in the full scan mass spectrum was identified as sodiated BDP. Upon CID, the sodiated BDP fragmented into product ions of *m/z* 507, 469, and 433 by the loss of hydrogen chloride, propionic acid, hydrogen chloride and propionic acid, respectively (Fig. 4b). In the MS^3 spectrum (the inset of Fig. 4b), the precursor ions (*m/z* 433) created the major fragments of *m/z* 415, 359, and 331 by the loss of water, propionic acid, propionic acid and carbon monoxide, respectively. These data confirmed that the beclomethasone dipropionate in the nasal aerosol drug preparation was detected successfully.

Binary mixture of methyl salicylate and diphenhydramine spray. The nanoEESI-MS method is also useful for simultaneous detection of multiple compounds in aerosol drugs. To demonstrate this capability of nanoEESI-MS, a binary mixture of methyl salicylate and diphenhydramine spray was rapidly analyzed to generate the nanoEESI mass spectrum (Fig. 5) using the nanoEESI-MS technique. The protonated diphenhydramine peak at *m/z* 256 is so abundant (1.33×10^6 cps) that the relatively weak signals from other compounds, such as the peak at *m/z* 153 with an abundance of 8.52×10^3 cps, are hardly seen in the same scale. However, in the zoomed view of the mass range between *m/z* 140 and *m/z* 160 (the inset of Fig. 5), the peaks for the protonated methyl salicylate and/or camphor at *m/z* 153, the protonated menthol at *m/z* 157, and the protonated thymol at *m/z* 151 can be clearly observed, respectively.

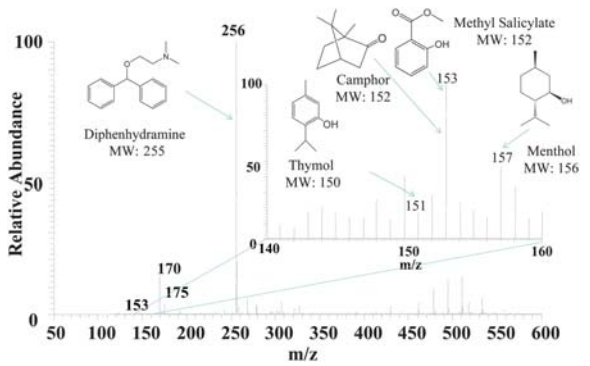


Fig. 5 NanoEESI mass spectrum of a binary mixture of methyl salicylate and diphenhydramine spray sample. The inset shows the zoomed view of the mass range between *m/z* 140 and *m/z* 160, and the signals of protonated thymol, protonated camphor, protonated methyl salicylate, and protonated menthol are simultaneously detected.

In general, nanoEESI is a softer ionization method compared to standard ESI,[57] but they often produce similar tandem mass spectra for small molecules.[22,37,53,56,58] In the CID spectrum as shown in Fig. S2,† the precursor ions of diphenhydramine (*m/z* 256) generated ions of *m/z* 167 and 88 as the major fragments by the loss of 2-dimethylamine ethanol ($HOCH_2CH_2N(CH_3)_2$) and diphenylmethane, respectively. All other assignments mentioned above were confirmed using the authentic compounds against the MS/MS data listed in Table 1. In the MS/MS spectrum of protonated thymol (*m/z* 151), the fragment ions at *m/z* 133, 136 and 109 could arise from the loss of H_2O, CH_3, and $CH_3CH{=}CH_2$ from the precursor ions (Fig. S3†). Protonated camphor ions (*m/z* 153) generated product ions of *m/z* 135, 125, 107, and 95 by the loss of H_2O, CO, ethanol, and acetone, respectively (Fig. S4b†). Protonated methyl salicylate ion (*m/z* 153) produced product ions of *m/z* 135 and *m/z* 121 by the loss of H_2O and methanol, respectively (Fig. S4c†). Note that methyl salicylate and camphor have different molecular structures but the same mass to charge ratio in the mass spectrum recorded using our instrument. However, these two isomers were differentiated by their CID spectral data, showing the potential capability of nanoEESI-MS/MS for differentiation of isomers (Fig. S4†). In the MS/MS spectrum of protonated menthol (*m/z* 157), the major fragments of *m/z* 139, 125, 115, and 99 were created by the loss of H_2O, CH_3OH, $CH_3CH{=}CH_2$, and CH_3COCH_3, respectively (Fig. S5†). Based on these experimental results, it can be concluded that nanoEESI-MS can be applied for profiling complex samples as well as identifying multiple important ingredients in aerosol drug preparations.

Table 1 Compounds detected from binary mixture of methyl salicylate and diphenhydramine spray by nanoEESI-MS/MS

Compounds	Observed ions		MS/MS product ions
	Ionic species	*m/z*	*m/z*
Diphenhydramine	$[M + H]^+$	256	167, 88
Thymol	$[M + H]^+$	151	136, 133, 109
Camphor	$[M + H]^+$	153	135, 125, 107, 95
Methyl salicylate	$[M + H]^+$	153	135, 121
Menthol	$[M + H]^+$	157	139, 125, 115, 99

Thermal degradation of salbutamol aerosol. Many drug compounds are sensitive to the environment. High throughput measurement is highly desirable for pharmacological studies, particularly for understanding the degradation and metabolism of drug compounds under given conditions. As a demonstration, nanoEESI-MS was used to study the thermal degradation process of salbutamol since it is sensitive to heat. As noted in Fig. 2b, salbutamol was easily detected as the protonated ion at *m/z* 240. In the mass spectrum of the well qualified salbutamol products, the only predominant peak detected was shown at *m/z* 240 (Fig. 2b). Once the aerosol drug was heated (≥80 °C) for a while (*e.g.*, >0.5 h), a new peak at *m/z* 86 was observed. The signal abundance of this peak (*m/z* 86) increased significantly when the salbutamol solution was heated for a longer time. Fig. 6a shows the nanoEESI-MS spectrum obtained using a salbutamol drug solution heated at 93 °C in water-bath for 4 h. In comparison with the spectrum shown in Fig. 2b, the signal abundances for the peaks at *m/z* 268 and *m/z* 86 were significantly increased. The peak at *m/z* 268 was identified as the product of salbutamol and ethanol.[17] The new peak at *m/z* 86 was interpreted as the major pyrolysis product of salbutamol (*i.e.*, $[(CH_3)_3CNHCH_2]^+$), which generated major fragments of *m/z* 69 and *m/z* 30 (the inset of Fig. 6a), might arise by the loss of NH_3 and $CH_2{=}C(CH_3)_2$, respectively. Thermal stability of

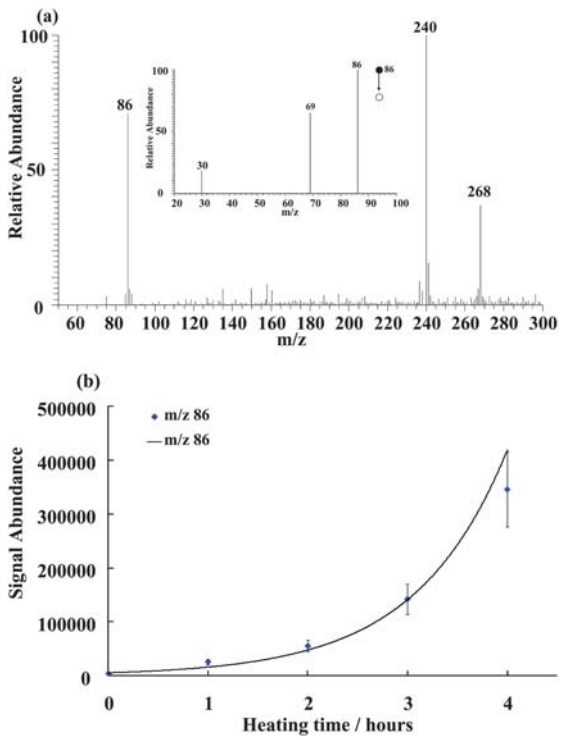


Fig. 6 Investigation of the thermal degradation kinetics of salbutamol using nanoEESI-MS. a): NanoEESI-MS spectrum of salbutamol aerosol drug heated for 4 h; The peak at *m/z* 86 was $[(CH_3)_3CNHCH_2]^+$ as the major pyrolysis product of salbutamol, and the inset shows the MS/MS spectrum of *m/z* 86; b): the dynamic decomposition of salbutamol induced by heat; the bar symbols designate the 15% of the mean values.

a drug is one of the most important properties for manufacturing, storage, and application. Fig. 6b shows the relationship between the heating time and the signal abundance of the decomposition product (*m*/*z* 86). From Fig. 6b, it can be fitted that the kinetic curve is $y = 5391.6e^{1.0884x}$ with the coefficient of determination $R^2 = 0.964$. The signal deviation became wider when the samples heated for a longer time were directly sprayed into the primary ion plume, probably because more pyrolysis products of salbutamol were created and thus distributed heterogeneously in the sample. However, it is clearly shown the signal abundance of the pyrolysis product (*m*/*z* 86) is higher when the heating time is longer. This agrees well with the thermal decomposition of the drug.[59] These findings show that nanoEESI-MS is a promising tool for instant studies of the dynamic drug degradation process, showing multiple applications in drug discovery and pharmaceutical regulation analysis.

High-throughput and potential quantitative capacities of nanoEESI-MS

NanoEESI has the capacity for high throughput analysis, which makes this technique highly efficient for fast screening of aerosol drugs on the market. By directly spraying aerosol samples into the ionic plume created by the gasless nanoESI, the ion chromatogram of the characteristic peaks showed instant responses to the presence of aerosol drug samples. The signal showed up to its 90% maximum within 3 scans when the aerosol sample was introduced into the nanoEESI source, and it dropped down to the background level within 3 scans once the sample was removed. As shown in Fig. 7a, the characteristic signals obtained from 5 different aerosol samples showed no serious carry-over effect for the sample analysis, probably because the sample container and the aerosol introduction tube were replaced for each sample. In this study, the sample-loading step took about 10 s for each sample using manual operation, providing a convenient way for fast analysis of aerosol drug preparations. Apparently, nanoEESI should be readily coupled to virtually any type of mass analyzer and can be easily implemented in a miniature mass spectrometer for *in situ* analysis since it requires no sheath gas (*i.e.* the heavy gas cylinder) for sample analysis. Therefore, nanoEESI-MS provides a practically convenient tool for instant and/or online screening.

In the present study, salbutamol aerosol was used to test the potential quantitative capacity of the nanoEESI-MS technique since it is a widely prescribed medication. Under the optimized experimental conditions, reproducible TIC signals were obtained for 13 measurements (Fig. 7b) using salbutamol aerosol drug samples, providing a reasonable RSD (6.39%, $n = 13$) for qualitative analysis. Note that a key step for reproducible results is to press the actuation trigger with full strength, and as fast as possible. The limit of detection (LOD) of this method was measured using the most abundant characteristic fragments of salbutamol observed in MS/MS experiments. Under the experimental conditions, the LOD was 10 ppt (S/N = 3) for the 100 μL salbutamol in ethanol solution sample. In general, internal standard is preferred for the actual quantification, especially in raw matrices. However, large amounts of drugs in raw matrices were inaccessible from the manufacturers in this project. Concentrations may change dramatically once the spraying

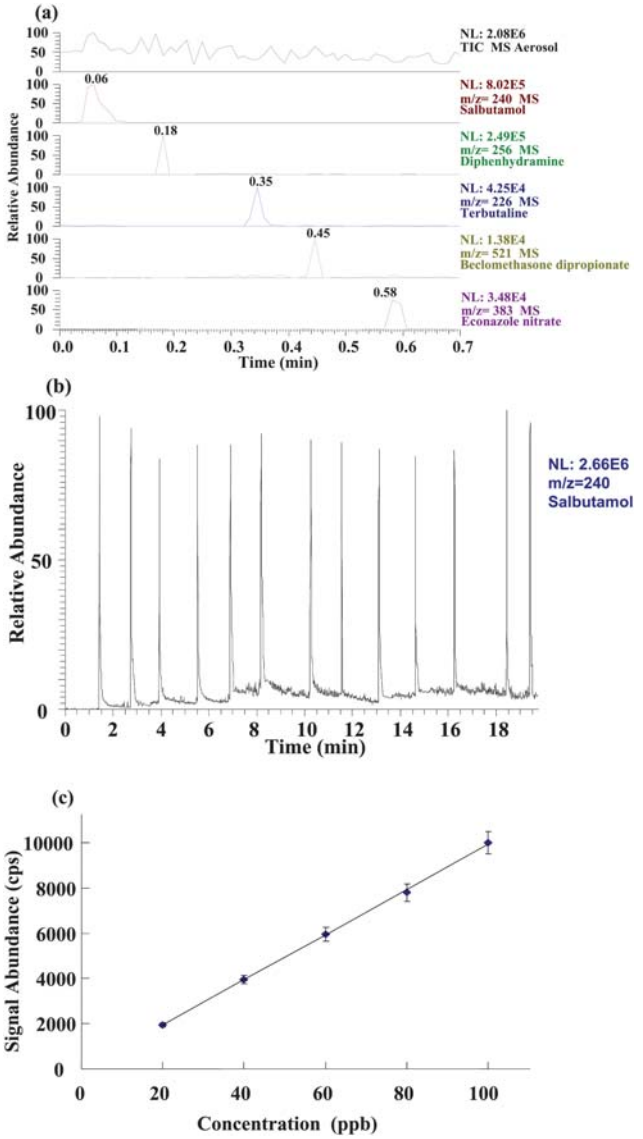


Fig. 7 High throughput analysis of multiple components of aerosol drug preparations. a): Selected ion traces of difference compounds detected at *m*/*z* 240, 256, 226, 521 and 383 using salbutamol aerosol, binary mixture of methyl salicylate and diphenhydramine spray, terbutaline sulfate aerosol, beclomethasone dipropionate nasal aerosol, and econazole nitrate spray, respectively. Each peak was recorded with an average time of 1.2 s; b): TIC traces of the protonated salbutamol (*m*/*z* 240) generated in the nanoEESI-MS experiments, providing a reasonable RSD (6.39%, $n = 13$); c): Signal responses of the characteristic fragment of protonated salbutamol (*m*/*z* 240) *vs.* the concentration of salbutamol ethanol solutions: $y = 0.9775x + 1.05$, $R^2 = 0.9989$.

bottles are opened due to the quick vaporization of components stored under high pressure. Noting the high tolerance of EESI to matrix effect, a series of standard salbutamol in ethanol solutions (20–100 ppb) were prepared to examine the linear dynamic range of nano-EESI-MS in the detection of active compounds in aerosol drugs. In Fig. 7c, signal responses of the peak at *m*/*z* 222 derived from the precursor ions of *m*/*z* 240, obtained by using nanoEESI-MS/MS with different concentrations of salbutamol ethanol solutions, can be fitted to $y = 0.9775x + 1.05$, $R^2 = 0.9989$. The acceptable RSD values and sensitivity achieved

from the salbutamol aerosol samples suggest nanoEESI-MS has great potential in quantitative analysis of complex samples such as pharmaceutical aerosol preparations.

Conclusion

The nanoEESI-MS device was successfully constructed and applied to the analysis of five commonly used aerosol drug samples including econazole nitrate spray, beclomethasone dipropionate nasal aerosol, binary mixture of methyl salicylate and diphenhydramine spray, terbutaline aerosol, and salbutamol aerosol. It has been demonstrated in this study that nanoEESI is a promising tool for the quantitative analysis of aerosol drug preparations with high throughput and high sensitivity. Active ingredients in each aerosol drug were detected within a few mass spectral scans, which took only a few seconds. The identifications of these compounds were confirmed by tandem mass spectrometry. By using salbutamol aerosol as the representative sample, nanoEESI-MS achieved a reasonably low RSD (6.39%, $n = 13$) for the rapid analysis of complex matrix samples. The LOD for salbutamol was found to be 10 ppt using the MS/MS spectrum. In addition, nanoEESI allows the experiments to be performed without any sheath gas or discharge gas, so the nanoEESI source can be coupled to portable mass spectrometers leading to *in situ* analysis without sample pretreatment. Thus nanoEESI has potential applications for fast screening inferior commercial products such as expired aerosol drug products on the market. The readiness of nanoEESI-MS to follow the degradation of drug compounds in a nearly real time fashion has also been demonstrated using salbutamol as the sample, showing promising applications in pharmacology studies.

Acknowledgements

This work was supported by the Innovation Method Fund of China (2008IM040400) and a grant from HIT (IMJQ10070004), and partly by a grant from MOST of China (2009 DFA41880).

References

1 H. Kataoka, *Curr. Pharm. Anal.*, 2005, **1**, 65–84.
2 C. K. Lim and G. Lord, *Biol. Pharm. Bull.*, 2002, **25**, 547–557.
3 A. G. Dragan, O. L. Cinteza, H. Weisssieker and P. Schaff, *Fresenius Environ Bull.*, 2009, **18**, 3–11.
4 E. H. Kerns and L. Di, *Curr. Drug Metab.*, 2006, **7**, 457–466.
5 M. J. Bogan, E. Patton, A. Srivastava, S. Martin, D. P. Fergenson, P. T. Steele, H. J. Tobias, E. E. Gard and M. Frank, *Rapid Commun. Mass Spectrom.*, 2007, **21**, 1214–1220.
6 W. D. Bennett, J. S. Brown, K. L. Zeman, S. Hu, G. Scheuch and K. Sommerer, *J. Aerosol Med.*, 2002, **15**, 179–188.
7 A. B. Watts, J. T. McConville and R. O. Williams, *Drug Dev. Ind. Pharm.*, 2008, **34**, 913–922.
8 J. Mitchell, S. Newman and H. K. Chan, *AAPS PharmSciTech*, 2007, **8**, 237–E12.
9 S. Willig, ed., *Good Manufacturing Practices for Pharmaceuticals: A Plan for Total Quality Control from Manufacturer (Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs)*, Informa HealthCare, London, 2000.
10 P. Begat, P. M. Young, S. Edge, J. S. Kaerger and R. Price, *J. Pharm. Sci.*, 2003, **92**, 611–620.
11 G. Buckton and H. Gill, *Adv. Drug Delivery Rev.*, 2007, **59**, 1474–1479.
12 H. K. Chan, *Colloids Surf., A*, 2006, **284–285**, 50–55.
13 A. J. Hickey, H. M. Mansour, M. J. Telko, Z. Xu, H. D. C. Smyth, T. Mulder, R. Mclean, J. Langridge and D. Papadopoulos, *J. Pharm. Sci.*, 2007, **96**, 5.
14 H. M. Mansour and A. J. Hickey, *AAPS PharmSciTech*, 2007, **8**, 140–E16.
15 A. New, D. Prime, S. Zomer, D. Elder, R. Donovan and E. Freney, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 3873–3882.
16 M. Careri, A. Mangia and M. Musci, *J. Chromatogr., A*, 1996, **727**, 153–184.
17 M. Li, B. Hu, J. Li, R. Chen, X. Zhang and H. Chen, *Anal. Chem.*, 2009, **81**, 7724–7731.
18 J. K. Kim, S. N. Jackson and K. K. Murray, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 1725–1729.
19 R. Flamini and M. De Rosso, *Expert Rev. Proteomics*, 2006, **3**, 321–331.
20 H. Chen, H. Liang, J. Ding, J. Lai, Y. Huan and X. Qiao, *J. Agric. Food Chem.*, 2007, **55**, 10093–10100.
21 S. Yang, H. Chen, Y. Yang, B. Hu, X. Zhang, Y. Zhou, L. Zhang and H. Gu, *Chin. J. Anal. Chem.*, 2009, **37**, 315–318.
22 L. C. Chen, Y. Hashimoto, H. Furuya, K. Takekawa, T. Kubota and K. Hiraoka, *Rapid Commun. Mass Spectrom.*, 2009, **23**, 333–339.
23 F. Zhang, Z. Jia, P. Gao, H. Kong, X. Li, J. Chen, Q. Yang, P. Yin, J. Wang, X. Lu, F. Li, Y. Wu and G. Xu, *Talanta*, 2009, **79**, 836–844.
24 M. Glinski and W. Weckwerth, *Mass Spectrom. Rev.*, 2006, **25**, 173–214.
25 G. A. N. Gowda, S. Zhang, H. Gu, V. Asiago, N. Shanaiah and D. Raftery, *Expert Rev. Mol. Diagn.*, 2008, **8**, 617–633.
26 R. G. Cooks, Z. Ouyang, Z. Takats and J. M. Wiseman, *Science*, 2006, **311**, 1566–1570.
27 Z. Takáts, J. M. Wiseman, B. Gologan and R. G. Cooks, *Science*, 2004, **306**, 471–473.
28 D. R. Ifa, N. E. Manicke, A. L. Rusine and R. G. Cooks, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 503–510.
29 H. Chen, N. N. Talaty, Z. Takáts and R. G. Cooks, *Anal. Chem.*, 2005, **77**, 6915–6927.
30 D. J. Weston, R. Bateman, I. D. Wilson, T. R. Wood and C. S. Creaser, *Anal. Chem.*, 2005, **77**, 7572–7580.
31 I. Cotte-Rodriguez, C. C. Mulligan and G. Cooks, *Anal. Chem.*, 2007, **79**, 7069–7077.
32 J. Y. Yew, R. B. Cody and E. A. Kravitz, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 7135–7140.
33 R. B. Cody, J. A. Laramee and H. D. Durst, *Anal. Chem.*, 2005, **77**, 2297–2302.
34 K. Kpegba, T. Spadaro, R. B. Cody, N. Nesnas and J. A. Olson, *Anal. Chem.*, 2007, **79**, 5479–5483.
35 C. Y. Pierce, J. R. Barr, R. B. Cody, R. F. Massung, A. R. Woolfitt, H. Moura, H. A. Thompson and F. M. Fernandez, *Chem. Commun.*, 2007, 807–809.
36 H. Chen, A. Venter and R. G. Cooks, *Chem. Commun.*, 2006, 2042–2044.
37 H. Chen and R. Zenobi, *Chimia*, 2007, **61**, 843–843.
38 J. Ding, H. Gu, S. Yang, M. Li, J. Li and H. Chen, *Anal. Chem.*, 2009, **81**, 8632–8638.
39 I. Cotte-Rodriguez, H. Hernandez-Soto, H. Chen and R. G. Cooks, *Anal. Chem.*, 2008, **80**, 1512–1519.
40 S. Yang, B. Hu, J. Li, J. Han, X. Zhang, H. Chen, Q. Liu, Q. Liu and J. Zheng, *Chin. J. Anal. Chem.*, 2009, **37**, 691–694.
41 M. Haapala, J. Pol, V. Saarela, V. Arvola, T. Kotiaho, R. A. Ketola, Sami Franssila, T. J. Kauppila and R. Kostiainen, *Anal. Chem.*, 2007, **79**, 7867–7872.
42 N. Na, M. Zhao, S. Zhang, C. Yang and X. Zhang, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 1859–1862.
43 Y. Zhang, X. Ma, S. Zhang, C. Yang, Z. Ouyang and X. Zhang, *Analyst*, 2009, **134**, 176–181.
44 J. D. Harper, N. A. Charipar, C. C. Mulligan, X. Zhang, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2008, **80**, 9097–9104.
45 Y. Liu, Z. Lin, S. Zhang, C. Yang and X. Zhang, *Anal. Bioanal. Chem.*, 2009, **395**, 591–599.
46 G. J. Van Berkel, S. P. Pasilis and O. Ovchinnikova, *J. Mass Spectrom.*, 2008, **43**, 1161–1180.
47 J. Dong, Y. H. Rezenom and K. K. Murray, *Rapid Commun. Mass Spectrom.*, 2007, **21**, 3995–4000.
48 H. Chen, M. Li, Y. Zhang, X. Yang, J. Lian and J. Chen, *J. Am. Soc. Mass Spectrom.*, 2008, **19**, 450–454.

49 K. Chingin, H. Chen, G. Gamez, L. Zhu and R. Zenobi, *Anal. Chem.*, 2009, **81**, 123–129.
50 K. Chingin, G. Gamez, H. Chen, L. Zhu and R. Zenobi, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 2009–2014.
51 Z. Zhou, M. Jin, J. Ding, Y. Zhou, J. Zheng and H. Chen, *Metabolomics*, 2007, **3**, 101–104.
52 H. Chen, A. Wortmann and R. Zenobi, *J. Mass Spectrom.*, 2007, **42**, 1123–1135.
53 H. Chen and R. Zenobi, *Nat. Protoc.*, 2008, **3**, 1467–1475.
54 H. Chen, B. Hu, Y. Hu, Y. Huan, Z. Zhou and X. Qiao, *J. Am. Soc. Mass Spectrom.*, 2009, **20**, 719–722.
55 H. Chen, Y. Sun, A. Wortmann, H. Gu and R. Zenobi, *Anal. Chem.*, 2007, **79**, 1447–1455.
56 H. Chen, A. Wortmann, W. Zhang and R. Zenobi, *Angew. Chem., Int. Ed.*, 2007, **46**, 580–583.
57 H. Chen, D. Touboul, M. C. Jecklin, J. Zheng, M. Luo and R. Zenobi, *Eur. J. Mass Spectrom.*, 2007, **13**, 273–279.
58 H. Chen, S. Yang, A. Wortmann and R. Zenobi, *Angew. Chem., Int. Ed.*, 2007, **46**, 7591–7594.
59 F. S. Felix, L. C. C. Da Silva, L. Angnes and J. R. Matos, *J. Therm. Anal. Calorim.*, 2009, **95**, 877–880.