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ARTICLE *in* ANALYTICAL CHEMISTRY · JANUARY 2006

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Direct Analysis of Pharmaceutical Drug Formulations Using Ion Mobility Spectrometry/ Quadrupole-Time-of-Flight Mass Spectrometry Combined with Desorption Electrospray Ionization

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A novel approach to the rapid analysis of pharmaceutical drug formulations using hyphenated ion mobility spectrometry (IMS) and time-of-flight mass spectrometry (ToF-MS) that requires no sample pretreatment or chromatographic separation is described. A modified quadrupole time-of-flight mass spectrometer containing an ion mobility drift cell was used for gas-phase electrophoretic separation of ions prior to ToF-MS detection. The generation of sample ions directly from tablets and cream formulations was effected by desorption electrospray ionization (DESI) using a modified electrospray ion source. The analysis of a range of over-the-counter and prescription tablet formulations is described, including histamine H_2 receptor antagonist (ranitidine), analgesic (paracetamol), opiate (codeine), and aromatase inhibitor anticancer (anastrozole) drugs. The successful determination of active drugs from soft formulations, such as an antiseptic cream (chlorhexidine) and a nicotine-containing skin patch, is also presented. Limits of detection for the active drugs using the DESI/IMS/ToF-MS method fell within the high-picomole to nanomole range. In all cases, the use of ion mobility drift tube separation showed increased selectivity for active drug responses (present as low as 0.14% w/w) over excipient responses such as poly(ethylene glycol). Tandem mass spectrometric analysis of precursor ions separated by IMS allowed positive confirmation of active drugs with little loss of ion mobility efficiency. The ability to analyze hard or soft pharmaceutical formulations directly by DESI combined with ion mobility spectrometry/mass spectrometry in ~2 min demonstrates the potential applicability of this novel method to pharmaceutical screening of low-molecular-weight drug formulations with high selectivity over the formulation vehicle.

Ion mobility spectrometry (IMS) is a gas-phase electrophoretic technique in which ions are separated on the basis of their relative mobilities, determined by the ion mass, charge, and collision cross section (i.e., size and shape). Many excellent reviews and tutorials exist detailing the fundamental principles and applications of ion mobility spectrometry,[1−4] an explanation of which falls outside the scope of this research article. The main advantages of the IMS technique are the speed, selectivity, and simplicity of the separation process, and these characteristics have resulted in the routine use of IMS in many diverse areas. The military arena has seen lightweight chemical agent detectors containing miniature ion mobility spectrometers being issued to troops on the battlefield.[5] In matters of national defense and security enforcement, IMS has become an indispensable tool.[6] Escalating requirements for screening of suspected nerve or chemical agents have seen use of IMS (or "electronic nose") systems around the world for trace-level detection of these agents by airport and government security staff.[7]

The use of ion mobility techniques is also increasing in the areas of biomedical, pharmaceutical, and environmental sciences. The use of IMS for tracking and diagnosing bacterial infections by rapid and selective detection of biogenic amine markers has shown the specificity and sensitivity possible with IMS as well as reduced the incidence of misdiagnosis.[8] The separation of pharmaceutical actives by atmospheric pressure IMS has been reported for the distinction of analogues based upon relative

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10.1021/ac051277q CCC: $30.25
Published on Web 10/26/2005

mobility.[9] Environmental monitoring of trace levels of hazardous compounds, such as explosives,[10] perfluorocarbons,[11] and volatile organic compounds (VOCs),[12] has been achieved in real time by IMS.

Hyphenated IMS techniques have attracted growing interest, and the combination of IMS with mass spectrometry (MS) brings the additional dimension of mass-to-charge (m/z) selectivity to the combined speed and selectivity of IMS. Hybrid IMS/MS has been applied to the detection of drugs of abuse[13] as well as fundamental studies, such as the elucidation of charge location on gas-phase peptides[14] and structural differences between cyclic and linear peptides.[15] Structural elucidation using hybrid IMS configurations has been reported for the analysis of amines[16] and noncovalent complexes[17] as well as polymers and biomolecular ions.[18] The use of IMS/MS as a new paradigm for proteomics which shows utility as a rapid means of two-dimensional separation of complex biological samples[19,20] has been proposed. The application of IMS/MS techniques in combinatorial chemistry,[21] where a library of ion-mobility and MS (m/z) data helped determine automatic failure or success in synthesis of tetrapeptides, demonstrates current innovative applications. Desorption ionization methods, such as matrix-assisted laser desorption ionization (MALDI), have been coupled with IMS[22] as well as in hybrid IMS/MS instruments[19,20] to effect ionization of solid samples in the presence of matrix.

Desorption electrospray ionization (DESI), introduced recently by Cooks et al.,[23] relies upon the formation of secondary ions from an analyte in the condensed phase using charged solvent droplets from a pneumatically assisted electrospray ion source. This seminal work showed that DESI/MS was able to desorb and detect compounds such as alkaloids from a plant stem cross section, drug molecules from in vivo sampling of living skin after ingestion of an antihistamine drug (loratadine), and explosives from a tanned leather surface with short analysis times and with no sample pretreatment or addition of matrix.

Explosives desorbed from a variety of surfaces have been analyzed successfully by Cooks and co-workers.[24] For example, TNT, RDX, PETN, and HMX were detected directly from paper, plastic, and metal surfaces at the low- to mid-femtogram level. Van Berkel et al. have demonstrated a novel DESI application[25] in which the direct analysis of thin-layer chromatography (TLC) plates was performed using DESI to desorb spatially resolved drugs from a series of developed TLC plates. By moving the plate relative to a DESI solvent plume, akin to moving the plate over a stationary flame ionization detector, desorption profiles were generated which contained either full scan or selected-reaction-monitoring (SRM) MS data. The application of this technique to the analysis of a solvent-extracted over-the-counter pain relief tablet, active drugs from which were separated using normal-phase TLC, showed rapid analysis times of ~4 min and detection limits comparable to previous hybrid TLC/MS systems from their group.

Although the mechanisms of DESI are not fully understood, some insights have been obtained into the impact of low kinetic energy cluster ions, generated from solvent, colliding with surfaces bearing organic or biological target analyte molecules.[24,26] It has been postulated that chemical sputtering is involved in the ionization process, along with electron-transfer, either during or in the gas-phase following desorption. The advent of DESI as an alternative desorption method has created much interest because no sample pretreatment is required, the technique is amenable to a wide range of analyte molecules and surface types, and because the degree of modification required for existing mass spectrometer hardware is not extensive.[23,24]

In this work, we describe the rapid and direct analysis of over-the-counter and prescription drugs with no sample preparation or chromatographic separation using hyphenated ion mobility/time-of-flight mass spectrometry (IMS/ToFMS) coupled with DESI sample introduction. The ability of DESI to analyze intact samples and desorb analyte ions from various surfaces makes it a well-suited technique for the analysis of drug formulations by ion mobility. Although the direct analysis of drug tablets by DESI/MS has been reported briefly in supplemental information by Cooks et al.,[27] this work utilizes electrophoretic separation of active drug responses from excipient responses by ion mobility (IMS), followed by single-stage or tandem (MS/MS) mass spectrometric detection. The novel coupling of IMS with DESI is discussed, together with experimental and MS hardware considerations for the analysis of both solid tablet and soft cream drug formulations.

EXPERIMENTAL

Chemicals. HPLC grade methanol, HPLC grade acetonitrile, and analytical reagent (AR) grade glacial acetic acid were purchased from Fisher Scientific (Loughborough, UK). Distilled and deionized water was obtained in-house using a Triple Red water purification system (Triple Red, Long Crendon, UK). Bradykinin (RPPGFSPFR) was obtained from Sigma Aldrich (Gillingham, UK). Zantac 75 tablets (GlaxoSmithKline, Stevenage, UK), Paracetamol and Codeine Pain Relief Tablets (Boots PLC,

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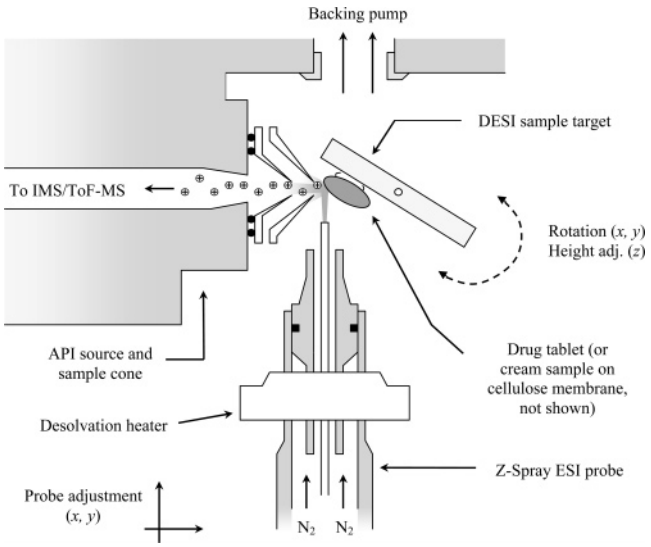

Figure 1. Schematic of DESI source and mass spectrometer interface.

Nottingham, UK), Nicorette Skin Patch (Pharmacia, Milton Keynes, UK), and Germolene antiseptic cream (Bayer AG, Leverkusen, Germany), were purchased over the counter. Arimidex tablets (AstraZeneca, Cheshire, UK) were obtained on prescription.

Solutions of 49/49/2 (v/v) acetonitrile/water/acetic acid or 49/49/2 (v/v) methanol/water/acetic acid were used as solvents in all DESI experiments. Aliquots of these solutions were also used to prepare stock solutions of bradykinin (120 μg/mL), in 49/49/2 (v/v) acetonitrile/water/acetic acid for initial evaluation of the DESI sampling method and sample plate setup.

DESI Source. The Z-Spray API source region of a Q-ToF Ultima mass spectrometer (Waters, Manchester, UK) was modified in-house for DESI analysis (Figure 1). A machined poly(tetrafluoroethylene) (PTFE) target plate (5.5 cm diameter) was polished with fine abrasive paper, mounted on a metal post, and positioned close to the mass spectrometer sample cone. The plate was held at an ~45° angle relative to both mass spectrometer sample cone and incident solvent plume from the ESI probe. All tablets were removed from their protective blister packaging using gloved hands (nitrile gloves) and attached to the target plate using a small quantity of nonvolatile adhesive before introduction into the ion source region. Soft cream formulations were spotted in small amounts (~5 mg, using a cotton-tipped safety swab) onto a cellulose nitrate filter membrane (0.65 μm pore size, 47 mm diameter, 125 μm thick, Whatman, Milton Keynes, UK), which was secured to the target plate using a minimal amount of double-sided tape, well away from the sample-to-solvent contact area.

IMS/Q-ToF/MS Instrumentation. Ion mobility and mass spectrometric data were obtained using a prototype low-pressure ion mobility quadrupole time-of-flight mass spectrometer (IMS-Q-ToF/MS)[28] (Figure 2) based upon a Q-ToF Ultima spectrometer (Waters, Manchester, UK) and controlled by custom-written MassLynx operating software (Waters, Manchester, UK). The Z-Spray API source region was modified to accept the DESI sample plate by overriding source interlocks and removing the glass source cover to give access for sample plate positioning. Care had to be taken to avoid exposure to the ESI high voltage and heated API source block. The probe used to create the DESI spray plume was a standard Z-spray electrospray ionization (ESI) probe, operated at 2.8−3.5 kV, with nebulizing gas (80 psi) and desolvation gas (250 L/h, 120−400 °C) being supplied from a nitrogen generator at 120 psi (Whatman, Milton Keynes, UK). Solvent was delivered from a glass syringe (500 μL, Gastight, Hamilton, Reno, NV) to the ESI probe by an integrated syringe pump (10−40 μL/min), regulated by the MS instrument software. Adjustment of the probe in the $x-y$ plane was carried out using existing manual screw controls that, in conjunction with $x-y-z$ control of plate position, gave adequate control of the sample relative to the mass spectrometer sample cone. Typical distances from the ESI sprayer tip to the sample (tablet or cream) and between the sample and the instrument sample cone were around 5−10 mm and 2−6 mm, respectively. These distances are similar to those detailed in other DESI applications.[23,25] Incident angles between the solvent plume and the target plate were around 45°, and care was taken to position the sample slightly off-axis relative to the outer sample cone to help prevent excess fouling of the inner sample cone. The approximate diameter of the solvent plume contact area on a solid sample was estimated to be around 6−8

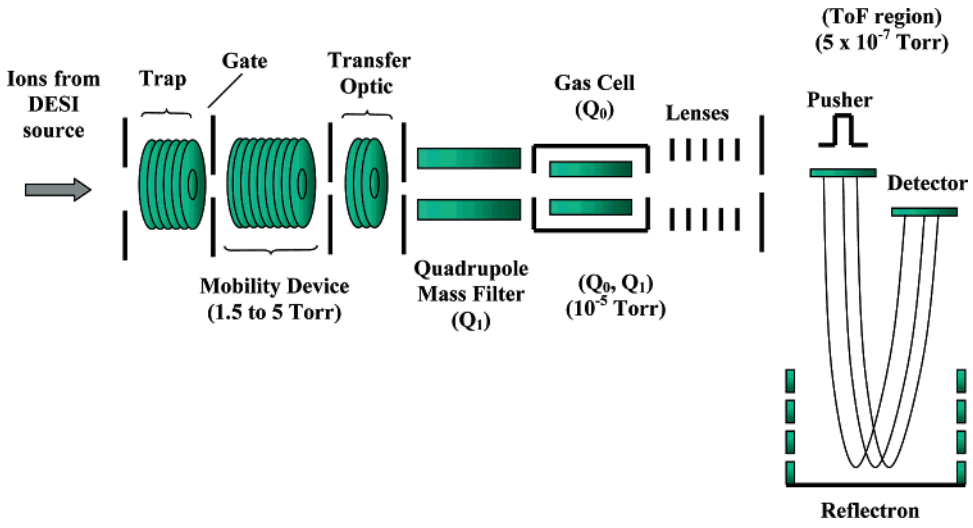

Figure 2. Schematic of hybrid IMS/Q-ToF/MS instrument.

mm^2, similar to a recent application of DESI in explosives analysis.[24]

Secondary ions sampled from the DESI target were directed via the MS sample cone (held at 120 °C) into the trap region at the head of the ion mobility drift cell (Figure 2), which was operated at a pressure in the region of 1.0−3.0 Torr. Ions were held radially by an RF ring-stack and axially by a trapping voltage (3.8 V) and injected into the drift cell using a gate electrode pulse (−4.95 V with respect to trapping voltage, 200-μs pulse width) at 15-ms intervals. The gate pulse was generated by a custom-made pulser unit (Waters, Manchester, UK) powered by a 240-V AC to 5-V DC step-down transformer. An RF ring-stack ion guide (15.2 cm long) to which a voltage gradient was applied (14.24 V/cm) was used as the ion mobility drift cell. A short RF ring-stack transfer optic was used to guide ions from the drift cell into the low-pressure region of the mass spectrometer. Radial confinement of ions traversing the drift cell and transfer lens was effected using superimposed RF voltages. An offset (1 V) and voltage gradient (1 V) were applied to the transfer optic using existing mass spectrometer RF generators powered by two external, low-ripple, DC power supplies (GP-4303D, EZ Digital Co. Ltd., Buena Park, CA).

The trap region and drift cell pressures were measured using a local Pirani gauge (APG-L-NW25, Edwards, Crawley, UK) controlled by the MassLynx software. The quadrupole mass filter (Q_1 in Figure 2) was operated in wide-band-pass mode, and an RF hexapole collision cell (Q_0 in Figure 2) was operated without collision gas (8−10 V collision cell energy) to transmit ions into the orthogonal acceleration ToF region. For tandem mass spectrometric experiments, the protonated drug precursor exiting the drift cell region was mass-to-charge-selected using the quadrupole mass filter and transferred into the collision cell (9−11 psi argon gas supply pressure, corresponding cell pressure of $\sim 5 \times 10^{-3}$ Torr, 18−23 V collision energy) for collision-induced dissociation (CID).

Single time-of-flight mass spectra were acquired at pusher intervals of 65 μs over a period of ~15 ms from the point of ion injection into the drift cell. Ion mobility spectra were processed automatically by collecting data from 200 sequential ToF pushes before the next injection into the drift cell. High-resolution m/z measurement was carried out over a maximum range of 100−1500, with typical resolution of 8500 at midrange m/z. Acquired data were presented as a plot of time (ToF scan number) against ion intensity (total ion mobility current or selected ion mobility current) using custom-written routines in the MassLynx software.

RESULTS AND DISCUSSION

Optimization of DESI and Detection of Secondary Ions. The in-house-constructed DESI source was evaluated initially using an aliquot (150 μL) of a solution of bradykinin (120 μg/mL, 113 pmol/mL) which was spotted onto the DESI target plate in the solvent-to-sample contact area. The spot size was ~6 mm in diameter (113 mm^2) with a surface concentration of 2.5 μg/mm^2 (2.4 nmol/mm^2) when dry. The sample plate was placed in the API source region, as shown in Figure 1, and sprayed with a solvent plume (49/49/2 (v/v) acetonitrile/water/acetic acid at 15 μL/min) from the ESI probe. The sample plate and ESI probe positions were adjusted to optimize secondary ion formation and detection of doubly charged bradykinin ions (m/z 530.21) desorbed from the target surface toward the mass spectrometer sample cone. Desolvation gas was used to assist desorption of secondary ions from the plate (N_2 gas flow 250 L/h, 180 °C).

The area of sample desorbed when the ESI probe and sample stage positions were optimized was ~7 mm^2 (1.5 mm diameter), which corresponded to the diameter of the incident ESI solvent plume at the sample surface. On the basis of the amount spotted, ~240 pg (226 fmol) of bradykinin was desorbed per ion mobility spectrum, giving an estimated limit of detection of 6.1 pg (5.7 fmol, 3:1 signal-to-noise). This experiment allowed the effect of the angles and distances between the ESI probe, the sample target, and the mass spectrometer sample cone to be investigated. Critical angles were found to be the angles between the incident solvent plume from the ESI probe and the sample target plate (~50°) and between the target plate and the mass spectrometer sample cone (~40°, just off direct line-of-sight to the cone), along with critical distances of probe to plate and plate to sample cone (~6 and 3 mm, respectively). The combined mass spectra from 200 ToF pushes (one complete ion mobility spectrum) would be expected to reflect the mass spectrum resulting from DESI analysis without ion mobility separation, and this forms the basis for a comparison of the DESI/IMS/MS technique with DESI/MS.

Analysis of Tablet Drug Formulations. All tablets were mounted directly onto the DESI target plate before introduction into the modified mass spectrometer API source region. No other sample pretreatment or preparation of the intact tablet was carried out.

Ranitidine (75 mg, Zantac 75 tablet, GlaxoSmithKline). A single Zantac 75 tablet (weighing 152 mg, containing 75 mg (238 μmol) ranitidine as active drug) was mounted onto the PTFE sample plate, placed in the API mass spectrometer source, and aligned for analysis as described previously. Solvent was sprayed at the intact tablet (49/49/2 (v/v) acetonitrile/water/acetic acid) at 10 μL/min, at a temperature of 180 °C with desolvation gas flowing at 250 L/h. Within 5−10 s, the pink coating of the tablet was removed, exposing an off-white inner surface, and an ion mobility response for ranitidine (m/z 315.2, $[C_{13}H_{22}N_4O_3S + H]^+$) was observed. Figure 3 presents the results of the ion mobility analysis of an intact Zantac tablet, showing the single ion mobility spectrum for m/z 315.2 (Figure 3a) and the total ion mobility spectrum for the whole scan range of m/z 200−1500 (Figure 3b). The arrow (scan 2000) signifies ion injection into the mobility cell. Ranitidine is observed as an extracted m/z 315.2 single ion mobility response with a peak maximum at scan 2031 (31 scans after ion injection into the drift cell) and a peak width at half-height of 6 scans and is much cleaner and sharper than the response shown in the total ion mobility spectrum. Likewise, when the mass spectrum obtained by combining scans across the ranitidine peak (scans 2029−2032, shown in Figure 3c) is compared to the mass spectrum from the combined total ion mobility spectrum (scans 2000−2200, Figure 3d), the greater selectivity of IMS over excipient responses is apparent. In Figure 3c, the resulting mass spectrum shows fewer excipient responses, due to active drug responses being electrophoretically resolved from the excipient

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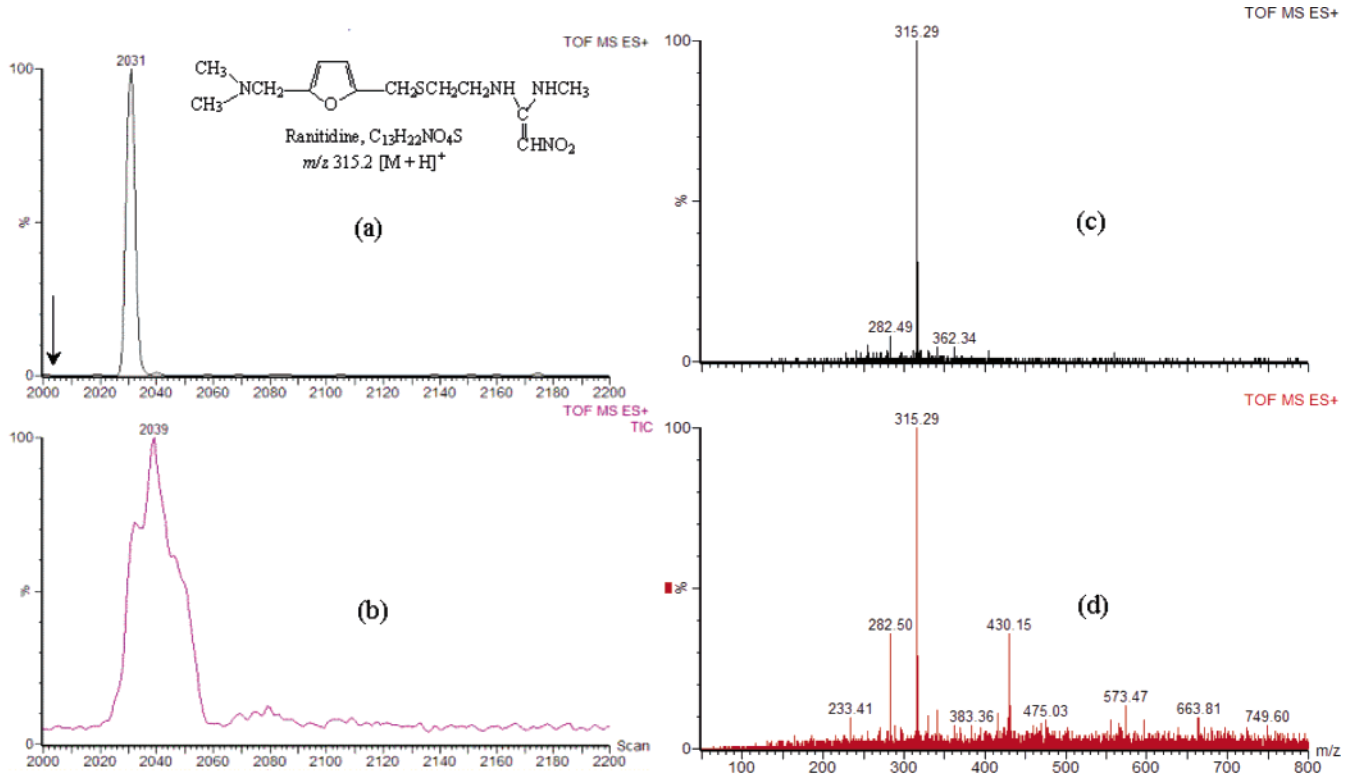

Figure 3. (a) Single ion mobility spectrum for ranitidine (*m*/*z* 315.2), (b) total ion mobility spectrum, (c) combined mass spectra from ranitidine IMS peak (scans 2029−2032), and (d) combined mass spectra from full IMS data (scans 2000−2200).

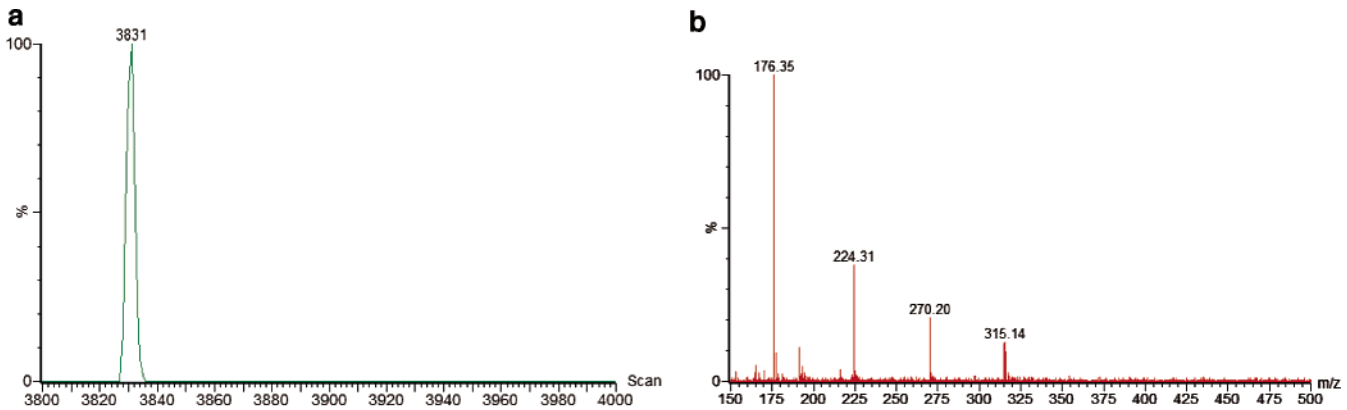

Figure 4. (a) Product ion mobility spectrum (*m*/*z* 176.3) from tandem mass spectrometry (MS/MS) of mobility-resolved ranitidine response and (b) combined product ion spectrum from the product ion mobility peak.

background observed in Figure 3d. Total acquisition time in this case was <2 min, accounting for a total of 0.2 mg of tablet being consumed. On the basis of the percentage of free-form active per tablet (49.3% w/w) and the number of mobility spectra acquired, this equated to a maximum of 5.8 μg (18 nmol) of ranitidine per mobility spectrum and a limit of detection for ranitidine of ~200 ng (630 pmol) at a signal-to-noise ratio of 3:1.

Positive confirmation of the active drug was carried out using tandem mass spectrometric (IMS/MS/MS) analysis of ion mobility responses of the parent drug, as detailed in the Experimental Section, for which *m*/*z* 315.20 was preselected using the quadrupole mass filter and additional collision gas (argon, 6 psi) and collision energy (23 V) were applied to the collision cell. Figure 4 shows the resulting IMS/MS/MS data, where the resulting extracted single ion mobility response for the *m*/*z* 176 fragment of ranitidine occurs at drift times coincident with the precursor (Figure 4a) and other fragment ions shown in the characteristic product ion spectrum (Figure 4b). The peak width of the product ion IMS peak is slightly greater than that of the precursor ion as a result of transmission through the gas-filled collision cell. These data show the applicability of the DESI/IMS/MS technique for rapid, intact tablet analysis and demonstrate that high quality structural data and discrimination against excipient responses are possible without the sample pretreatment or chromatographic separation required by alternative techniques.[29,30]

Anastrozole (1 mg, Arimidex, AstraZeneca). The active drug in Zantac was present at almost 50% total tablet weight, so the analysis of a tablet in which the active drug was at a much lower level (w/w) was investigated. Arimidex contains 1 mg of anastrozole, a nonsteroidal aromatase inhibitor used for the treatment

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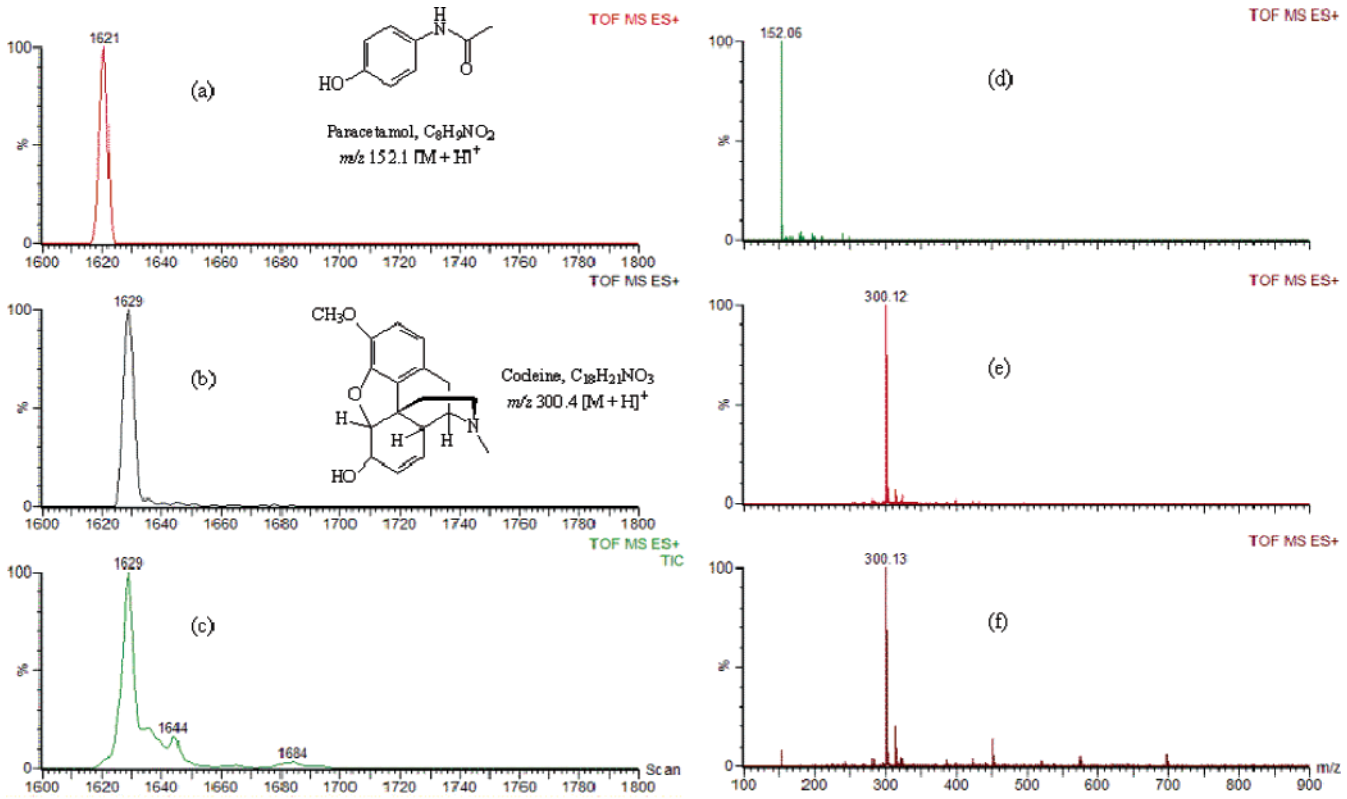

Figure 5. (a) Single ion mobility spectrum for paracetamol (*m*/*z* 152.1), (b) single ion mobility spectrum for codeine (*m*/*z* 300.1), (c) total ion mobility spectrum, (d) combined mass spectra from paracetamol IMS peak, (e) combined mass spectra from codeine IMS peak, and (f) combined mass spectra from full IMS data.

of estrogen-receptive breast cancer in post-menopausal women and has been shown to be effective in lowering selectively serum estradiol concentrations.[31] An Arimidex tablet (weighing 105 mg, 1 mg anastrozole (3.4 μmol) as active drug) was analyzed in a fashion similar to the Zantac tablet, above, using the same DESI solvent composition delivered at 15 μL/min.

The single ion mobility spectrum for anastrozole (*m*/*z* 294.38, $[C_{17}H_{19}N_5 + H]^+$) showed a sharp peak similar to that observed for ranitidine, with a peak maximum at scan 29, whereas the total ion mobility spectrum was much broader, showing a number of excipient responses. The Supporting Information details combined mass spectral data from the single ion mobility peak, which shows few excipient peaks, as compared to the combined mass spectrum from the total ion mobility spectrum. A repeating series (*m*/*z* 703 and 747, repeat *m*/*z* δ 44) from poly(ethylene glycol), present as the binding agent macrogol, dominated the higher mass range along with excess spectral noise in the midrange. Some lower mass excipient responses were present in the mobility-resolved mass spectrum but were not resolved completely from the anastrozole peak. Each ion mobility spectrum represented an average consumption of 3.1 μg (10.3 nmol) of anastrozole, which equates to a limit of detection of 210 ng (700 pmol) at a signal-to-noise ratio of 3:1, on the basis of tablet weight consumed and percent free-form active drug per tablet (0.95% w/w).

Paracetamol and Codeine (500 mg and 8 mg, Pain Relief Tablets, Boots PLC). The analysis of a dual-active formulation was performed on an over-the-counter pain relief tablet to evaluate the ability of IMS/MS to resolve actives from excipient responses and each other. The tablet contained paracetamol (acetaminophen, 500 mg, $[C_8H_9NO_2 + H]^+$, *m*/*z* 152.1) and codeine (8 mg, $[C_{18}H_{21}NO_3 + H]^+$, *m*/*z* 300.1) as active drug components and was analyzed using 49/49/2 (v/v) methanol/water/acetic acid (15 μL/min, with a desolvation temperature of 120 °C) as DESI solvent because of the high alcohol-solubility of both active drugs. Responses for both protonated active drugs were observed within seconds of starting the analysis after the surface coating of the tablet was stripped away by the electrosprayed solvent plume. Figure 5 shows the single ion mobility spectra for paracetamol (*m*/*z* 152.1, Figure 5a) and codeine (*m*/*z* 300.1, Figure 5b) and the total ion mobility spectrum for the tablet (Figure 5c). A close-to-baseline separation is attained for the mobility responses for paracetamol and codeine (peak maximums at scans 21 and 29, respectively). The mass spectra obtained by combining data from beneath the single ion mobility responses for each analyte are shown in Figure 5. Figure 5d shows the mass spectrum resulting from combining data from paracetamol response; Figure 5e, the spectrum from codeine response; and Figure 5f, the spectrum from the total ion mobility response.

Paracetamol is present in the tablet in large excess (over 60 times that of codeine (w/w)), although the relative responses for the two active drugs differ greatly. Differences in chemical structure and increased number of possible protonation sites lead to enhanced ionization efficiency for codeine, as compared to paracetamol in the positively charged solvent environment. The respective amount of active drug represented by a single ion mobility profile was 66 μg (434 nmol) and 1.1 μg (3.6 nmol) for paracetamol and codeine, respectively.

(31) Jonat, W. *Breast* **1996**, *5*, 209.

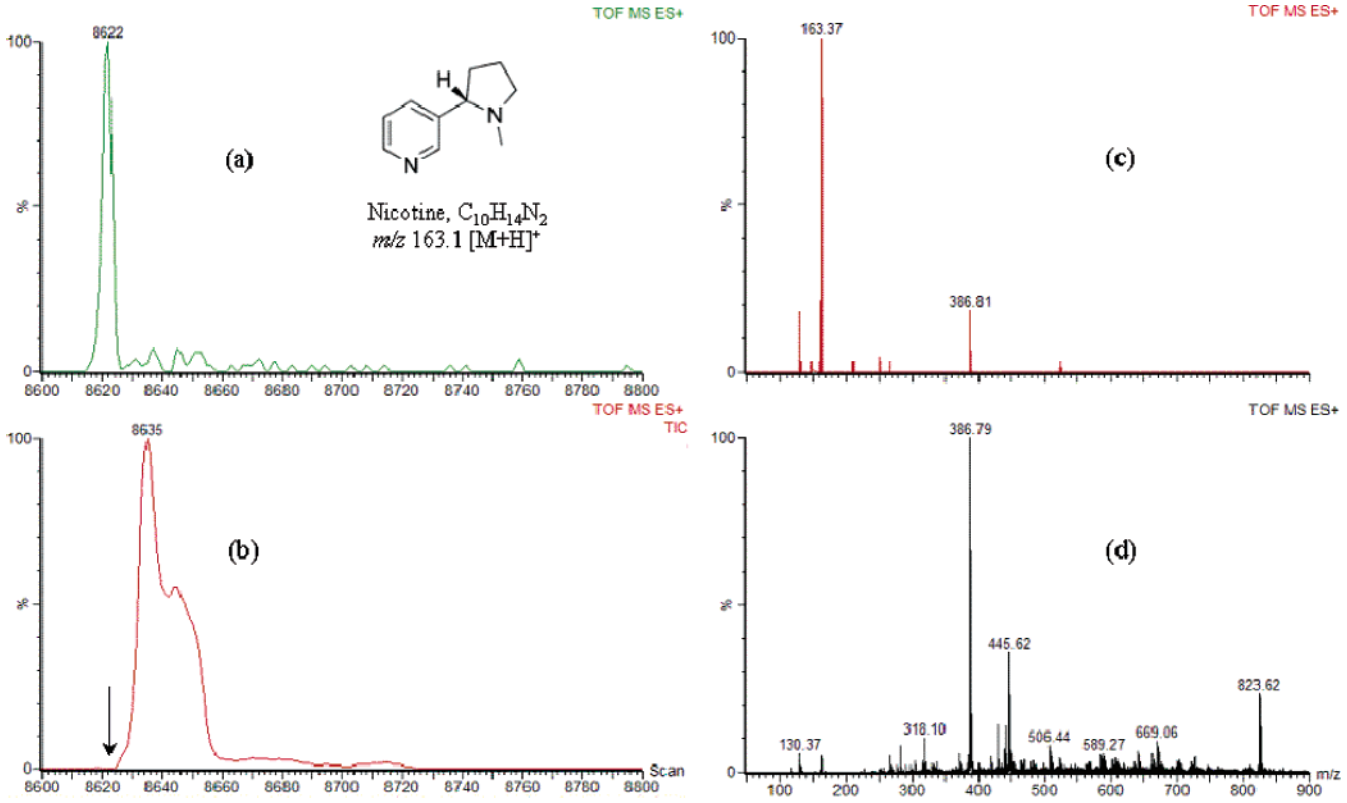

Figure 6. (a) Single ion mobility spectrum for nicotine (*m/z* 163.4), (b) total ion mobility spectrum, (c) combined mass spectra from nicotine IMS peak, and (d) combined mass spectra from full IMS data.

Analysis of a Slow-Release Skin Patch. *Nicotine (24.9 mg, Nicorette Skin Patch, Pharmacia)*. A single Nicorette skin patch containing nicotine (24.9 mg total (153 μmol), 0.83 mg/cm^2 (5.1 μmol/cm^2)) and releasing 15 mg (92.4 μmol)/16 h) was mounted onto the DESI sample target using a small piece of double-sided tape. The patch was constructed of a drug vehicle (low-to-medium weight polyisobutylene) containing nicotine sandwiched between nonwoven polyester backing film (external patch surface) and a siliconized polyester drug release liner (with adhesive layer to adhere patch to the skin and affect drug transport).

Encouraging diffusion of nicotine (a low-molecular-weight, nonpolar compound) from the drug vehicle to the membrane surface and subsequent permeation through the nonpolar siliconized membrane liner was required for the analysis. By use of a combination of heat (desolvation gas flow 250 L/h, 380 °C) and a high flow of a suitable DESI solvent to wet the membrane surface (49/49/2 (v/v) acetonitrile/water/acetic acid, 40 μL/min), the active drug was successfully analyzed directly from the patch surface.

The selected ion mobility response for protonated nicotine (*m/z* 163.4, $[C_{10}H_{14}N_2 + H]^+$) shows a narrow mobility response for nicotine (peak maximum at scan 22, Figure 6a). In comparison, the total ion mobility spectrum (Figure 6b) shows a high response for excipient compounds, and the nicotine mobility response is barely distinguishable in the baseline (see arrow). However, the mass spectrum resulting from combined scans (fwhm) of the nicotine ion mobility peak (Figure 6c) shows the base peak as the nicotine parent ion (*m/z* 163.37) with few other responses, whereas the relative intensity of nicotine in the mass spectrum from the total ion mobility spectrum (Figure 6d) is low (~5%) as compared to overwhelming responses from patch excipients.

On the basis of the manufacturer-stated concentration of nicotine per unit area (0.83 mg/cm^2, 5.1 μmol/cm^2), the approximate area of direct solvent contact (~5 mm diameter, 0.79 cm^2), and the number of scans acquired, a single ion mobility spectrum was estimated to be observed from 15 μg (92 nmol) nicotine desorbed from the patch. The combination of ion mobility with DESI sample introduction along with a high temperature and high solvent flow (MIMS-type) approach has afforded the direct and selective analysis of nicotine from a complex delivery device with no prior pretreatment.

Analysis of a Soft Drug Formulation. *Chlorhexidine Gluconate (0.25% w/w, Germolene Antiseptic Cream, Bayer AG)*. An attempt to analyze an aliquot of Germolene cream deposited directly onto the PTFE target resulted in poor sensitivity and ion beam stability, with a transient and noisy response for chlorhexidine. A thin layer of Germolene cream (5 mg, containing 12.5 μg (14.0 nmol) chlorhexidine gluconate, $C_{22}H_{30}N_{10}Cl_2{\cdot}2(C_6H_{12}O_7)$) was therefore applied to a cellulose nitrate membrane filter attached to the DESI sample plate, as described previously. The desolvation temperature was increased to 400 °C (gas at 250 L/h), with DESI solvent (49/49/2 (v/v) acetonitrile/water/acetic acid) delivered at 15 μL/min. The physical force of the solvent plume striking the cellulose membrane initially removed some of the surface cream; however, adequate sample was retained by the porous cellulose substrate to give a sustained ion mobility responses for the active drug chlorhexidine (*m/z* 505.21, $[C_{22}H_{30}N_{10}Cl_2 + H]^+$) and good quality mass spectral data (Figure 7). The force of the DESI plume left a slight indentation on the surface of the cellulose membrane, which made visualization of the analyzed areas easier. The single ion mobility spectrum for *m/z* 505.2 obtained from analysis of a Germolene cream sample (Figure 7a)

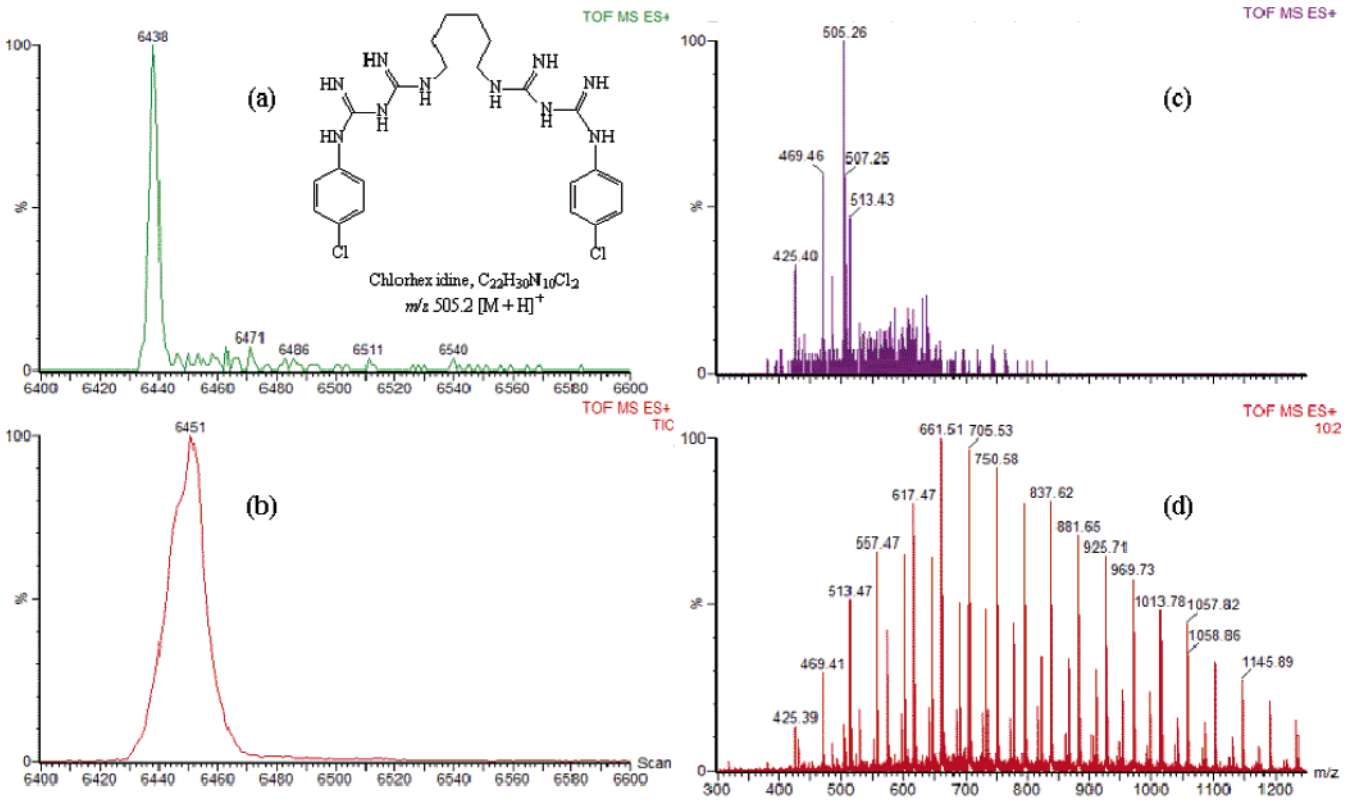

Figure 7. (a) Single ion mobility spectrum for chlorhexidine (*m*/*z* 505.3), (b) total ion mobility spectrum, (c) combined mass spectra from chlorhexidine IMS peak, and (d) combined mass spectra from full IMS data.

demonstrates a clean response for chlorhexidine (peak maximum at scan 6438). The total ion mobility spectrum (scans 6400−6600, Figure 7b) shows a single, broad, complex peak resulting from the excipient responses. The mass spectrum from the chlorhexidine single ion mobility peak (Figure 7c) shows the characteristic base peak at *m*/*z* 505.2 along with expected chlorine isotope peaks at 507.2 and 509.2. The mass spectrum from the total ion mobility peak (Figure 7d), in contrast, is dominated by peaks associated with poly(ethylene glycol) (PEG 800), a major inactive cream ingredient (vehicle). The response for chlorhexidine cannot be readily distinguished from the background due to PEG 800 in this spectrum. From the total amount of cream spotted (5 mg) and the amount of active (free-form) drug present (7.0 μg, 13.9 nmol, salt-factor of 1.777), an average ion mobility profile equated to around 159 ng (316 pmol) of active drug. The limit of detection for chlorhexidine in a Germolene cream matrix was estimated to be 25 ng (50 pmol) on the basis of total consumption of the cream and a signal-to-noise ratio of 3:1. Additional structural information for chlorhexidine was obtained via tandem mass spectrometric detection (MS/MS) of resolved ion mobility responses, in a way similar to that of the MS/MS routines applied to the Zantac 75 tablet, detailed previously. In this case, collision energy of 21 V was used, and argon gas was added to the collision cell at 9 psi.

Automatic isotopic-pattern recognition (or "isotopic strip") data interrogation methods were also applied to full-scan mobility data from the analysis of a Germolene cream sample (via the MassLynx software) to increase specificity and simplify the complex data set. Total ion mobility data were interrogated, searching for characteristic responses that satisfied the relative isotopic intensities and mass δ of a chlorinated ion (^{35}Cl at 100%, ^{37}Cl at 60%, mass δ 1.997) to within specified tolerances.[32] This demonstrates the ability to exploit characteristic properties of a given analyte and shows that in cases in which a combination of temporal resolution (IMS) and *m*/*z* information (MS) may not be sufficient, rapid and selective data interrogation may still be carried out.

CONCLUSION

This work has demonstrated a new approach for the direct analysis of pharmaceutical drug formulations using hyphenated ion mobility spectrometry/time-of-flight mass spectrometry combined with desorption electrospray ionization that requires no sample pretreatment or prior chromatographic separation. Ion mobility drift cell separation of desorbed ions combined with mass analysis using a hybrid quadrupole time-of-flight mass spectrometer shows significantly enhanced selectivity, as compared to direct MS analysis, and the generation of sample ions from tablets or cream formulations by DESI has demonstrated the novel utility of this desorption method combined with IMS.

The successful analysis of several hard or soft drug formulations ranging from an aromatase inhibitor anticancer drug to an antiseptic cream has been presented. Limits of direct detection for active drugs by DESI/IMS/Q-ToF/MS fell within the high-picomole to nanomole range. In all cases, the use of electrophoretic separation by ion mobility showed increased selectivity for active drug responses (present as low as 0.14% w/w) over excipient responses, such as poly(ethylene glycol). Tandem mass spectrometry of mobility-resolved ions allowed positive confirmation of active drugs with little impact on the efficiency of the ion

(32) Supplemental information, available on-line from ACS site, linked to this paper.

mobility analysis. The ability to analyze hard and soft formulations directly in less than 2 min demonstrates the potential applicability of this novel method to pharmaceutical screening and development of drug formulations, with high selectivity over the formulation vehicle. The high-quality MS/MS data obtained using this rapid and selective method also show potential for the determination of unknown substances in areas such as drug authenticity screening.

ACKNOWLEDGMENT

This research was carried out under the UK National Initiative in Ion Mobility Spectrometry, a consortium supporting research into the development and application of ion mobility spectrometry at Nottingham Trent University and the University of Manchester, supported by AstraZeneca, GlaxoSmithKline, and Waters Corporation. The authors acknowledge Kevin Giles and Jason Wildgoose (Waters MS Technologies Centre, Manchester, UK) for helpful discussions regarding instrument modification.

SUPPORTING INFORMATION AVAILABLE

Data from analysis of Arimidex (anastrazole) tablet: (a) single ion mobility spectrum for anastrazole, (b) total ion mobility spectrum, (c) combined mass spectrum from anastrazole IMS peak, and (d) combined mass spectra from full IMS data. Data from cluster strip of Germolene (chlorhexidine) cream: (a) total ion mobility response following isotopic pattern recognition ("cluster strip") of chlorhexidine response, as compared to (b) uninterrogated total mobility response, (c) resulting mass spectrum from interrogated (stripped) ion mobility data. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review July 19, 2005. Accepted September 21, 2005.

AC051277Q