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Desorption Electrospray Ionization Mass Spectrometry for High-Throughput Analysis of Pharmaceutical Samples in the Ambient Environment

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Desorption electrospray ionization (DESI) allows mass spectrometry to be used for on-line high-throughput monitoring of pharmaceutical samples in the ambient environment, without prior sample preparation. Positive and negative ion DESI are used to characterize the active ingredients in pharmaceutical samples formulated as tablets, ointments, and liquids. Compounds of a wide variety of chemical types are detected in these complex matrices. The effects on analytical performance of operating parameters, including the electrospray high voltage, heated capillary temperature, solvent infusion rate, and solvent composition, are evaluated and optimized. In addition to experiments in which a simple solvent is sprayed onto the solid analyte samples, reactive desorption is performed by adding reagents to the solvent spray to generate particularly stable or characteristic ions with the analytes of interest. A variable-speed moving belt was built for high-throughput sampling and used to provide rapid qualitative and semiquantitative information on drug constituents in tablets. Sampling rates as high as 3 samples/s are achieved in the ambient environment. Relative standard deviations of the relative ion abundances for major components in the mass spectra are in the range of 2−8%. Impurities and components present at levels as low as ~0.1% are identified and carryover effects are minimized in high-throughput on-line analysis of pharmaceutical samples.

High-throughput measurements are increasingly sought in drug discovery,[1−5] proteomics,[6−8] clinical diagnostics,[9,10] biochemical assays,[11] environmental monitoring,[12] combinatorial chemistry,[13] and food safety testing.[14,15] Techniques widely used in high-throughput analysis include near-infrared reflectance spectroscopy, UV−visible absorption spectroscopy, fluorescence spectroscopy, and, to a lesser extent, mass spectrometry. Raman and near-IR spectroscopies allow high-throughput analysis without the need for sample manipulation. They provide useful if somewhat limited information on the chemical composition of pharmaceuticals, in a nondestructive fashion.[16,17]

Mass spectrometric methods have traditionally involved considerable sample manipulation including extraction and chromatographic separation. For this reason, and also because samples are examined sequentially, high-throughput applications are not well developed. However, this is changing with the widespread use of automatic sample-handling equipment and the beginning of multiplexed or partially multiplexed instruments.[2,9,10,18−24] One approach, the MUX technology,[25] meets these demands by

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multiplexing four sample streams into a single mass spectrometer. The ability to couple parallel LC analyses with electrospray MS affords the opportunity to accelerate the rate of compound screening. This example typifies the present situation, which is that multiplexing of the separation but not the mass analysis step is performed. This approach works well when analysis rates are limited, not by the mass spectrometer, but by either the chromatography step or the sample introduction process. Thus, when a traditional analytical chromatography/mass spectrometry system is turned into a high-throughput system, the mass spectrometer is typically left unchanged. Spurred by the growing demand for MS and lab-on-a-chip technologies in life sciences research and in drug discovery, multiple nano LC columns have been combined with an electrospray chip.[26] A high-density ESI chip with 400 nanoelectrospray nozzles allows high-throughput analysis of protein samples.

Due to its ability to ionize spatially separated samples on solid surfaces, MALDI is probably even more widely used for high-throughput analysis than is ESI. Common examples include quality control of synthetic oligonucleotides and peptide mass fingerprinting. Automatic sample handling has been achieved using a specially designed interface between a microfluidic chip and a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer.[27]

The above examples of high-throughput work do not cover in situ analysis, which is of growing interest for environmental monitoring, quality control in manufacturing, and screening of biological samples in a clinical or related setting. In situ measurements have the potential to further increase the demand for suitable high-throughput mass spectrometry methods. The emergence of smaller portable mass spectrometers[28−34] is increasing the prospects for future in situ monitoring by mass spectrometry and hence the need for fully multiplexed instruments.

The recently developed technique of desorption electrospray ionization (DESI)[35] allows samples to be examined in the ambient environment. The method is relatively simple to implement and allows in situ and in vivo measurements while retaining the high sensitivity and specificity that characterize mass spectrometry. In addition, ions can be generated directly from solid surfaces of various types,[36] including the pharmaceutical preparations of interest in the present study. For all these reasons, the present study was undertaken to evaluate the use of DESI in high-throughput MS measurements.

The DESI method employs a pneumatically assisted electrospray which is used to impinge ionized solvent droplets and molecules onto a surface bearing the sample. The application area of DESI overlaps with that of MALDI,[37,38] the best established high-throughput method in mass spectrometry. However, DESI does not require prior surface modification (e.g., deposition of matrix compounds); hence, it can be applied for the interrogation of ordinary surfaces, including native biological surfaces and even living organisms.[35,39] The application area of DESI and the capability to examine natural surfaces in the environment overlap with that of the newly described direct analysis in real-time method.[40]

As an atmospheric ionization technique, DESI should be an excellent choice for high-throughput analysis. Noncoated tablets of over-the-counter drugs were chosen as model systems for initial tests of the capabilities of DESI for pharmaceutical analysis in a high-throughput mode. The coating of a coated tablet can be removed by the DESI spray at high solvent flow rates, or alternatively, physical abrasion of the tablet can be coupled with DESI since it is an ambient method. There are numerous mass spectrometric methods used for analysis of pharmaceutical preparations, both for quality assurance and for forensic purposes, but they start with analyte extraction or with dissolution of the tablet or other significant preparation step. Such processes are not only destructive but also time-consuming.

Like other desorption/ionization methods (MALDI, SIMS, etc.), DESI is essentially a minimally invasive analytical method, sample consumption being limited to subnanogram quantities, which is negligible in most cases.[35] The present study includes an investigation of the various factors influencing carryover effects in DESI mass spectrometry and demonstrates the feasibility of high-throughput analysis of some representative pharmaceutical preparations.

EXPERIMENTAL SECTION

Experiments were carried out using a Thermo Finnigan LTQ (San Jose, CA) mass spectrometer fitted with a home-built DESI source described elsewhere[35] and using the same experimental optimized parameters, unless otherwise noted. For single-sample analysis, the samples were placed onto a 3D moving stage (Newport, Irvine, CA) in order to optimize the sample position for analysis. As in previous experiments, the position of the DESI spray tip, the surface of the sample, and the front end of the heated capillary of the LTQ were optimized using a second moving stage. Liquid samples and ointments were examined after deposition of the analyte onto common filter paper. The paper carrying the samples was then attached onto the 3D moving stage for single-sample analysis. The operating parameters of the DESI source and those of the associated Finnigan LTQ mass spectrometer are the same as those reported previously.[35]

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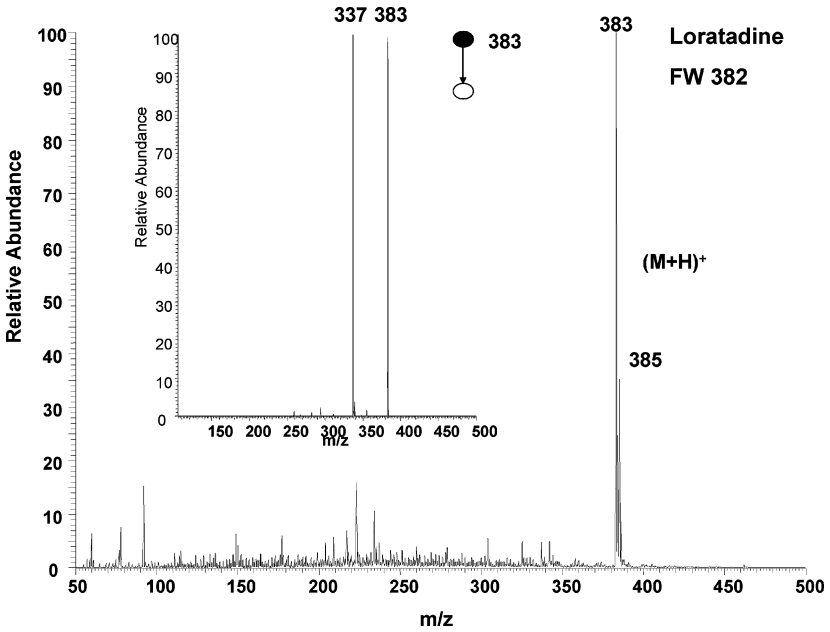

Figure 1. DESI-MS spectrum and product ion MS/MS spectrum of *m/z* 383 (inset) of Claritin tablet using methanol/water (1:1) as spray solvent in the positive ion detection mode.

When multiple samples were to be analyzed, a stepper motor with a 120-speed controller was used to run a moving belt at various speeds to simulate a production line in the pharmaceutical industry. The home-built transport system was constructed using two timing pulleys (1.5 in. in diameter), which were connected by a timing belt. One of the pulleys was powered by the stepper motor. All the parts were fixed tightly onto a metal base, which was then coupled to the LTQ-MS for high-throughput applications. Speeds varying from 0.1 to 15 tablets/s could be achieved. Using an adhesive copper tape (3M, St. Paul, MN), solid tablets were secured onto the plastic belt and electrosprayed droplets from the desorption electrospray ionization source were allowed to impact the surface of the sample. Several tablets could be placed at an equal distances along the moving belt, and the speed of analysis could be easily controlled.

Over-the-counter tablets (Claritin, folic acid, acetaminophen, aspirin, melatonin, Excedrin, Centrum), ointments (clotrimazole, ketoconazole), and liquid samples (Bausch & Lomb eye drops) were obtained from local pharmacies. Solvents (methanol, ammonium hydroxide, acetic acid, acetonitrile) were purchased from Mallinckrodt (Hazelwood, MO). All reagents were used without further purification. All dilutions requiring the use of deionized water were carried out by using the Barnstead Mega-Pure system D1 (Barnstead/Thermolyne, Dubuque, IA).

RESULTS AND DISCUSSION

Solid Tablets in the Positive Ion Mode. Experiments were done either by using a 3D moving stage to analyze a single tablet or by transporting a set of tablets located on a moving belt past the electrospray. The results discussed in this section were carried out using a single tablet in order to investigate the optimum operating parameters of DESI for such an experiment. Claritin, a popular antihistamine, gives a very simple mass spectrum with a characteristic chlorine isotopic signature, and hence, it was chosen to investigate the basic characteristics of DESI for analysis of active ingredients in pharmaceutical tablets prior to setting up the high-throughput experiments. Acetaminophen and aspirin were also used, and the results obtained were analogous. Figure 1 shows the positive ion DESI spectrum of Claritin recorded by using methanol/water (1:1) as the spray solution. It is dominated by the signal due to the active agent loratadine, ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*] pyridin-11-ylidene)-1-piperidinecarboxylate, the protonated molecule occurring at *m/z* 383 and its ^{37}Cl isotope peak appearing at *m/z* 385. This assignment was confirmed by tandem MS (inset in Figure 1) fragmentation being dominated by loss of CH_3CH_2OH from the ethyl ester side chain.

The effects of various experimental parameters, including the applied high voltage, the heated capillary temperature, the solvent infusion rate, and the solvent composition, were all examined using Claritin tablets as a representative case. The first three parameters have strong effects on the signal intensity for protonated loratadine ions. The other parameter, the solvent composition, showed only a slight effect on signal intensity. Results of experiments that investigated the dependence of signal intensity on the electrospray high voltage, capillary temperature, and solvent flow rate are shown in Figure 2. Interestingly, signal was observed even in the absence of an electrospray high voltage, viz. under sonic spray[41] conditions; however, the intensity was 2 orders of magnitude lower than that at the optimum ESI voltage. The signal intensity increased continuously with increasing high voltage leveling off at ~4 kV. In the absence of a high voltage, the ESI source[42] operates as a sonic spray ion source,[41] a process known to produce charged droplets. These charged droplets, like those formed in ESI, desorb molecules of the analyte during the collision (droplet pickup mechanism). Ionization may also occur by heterolytic

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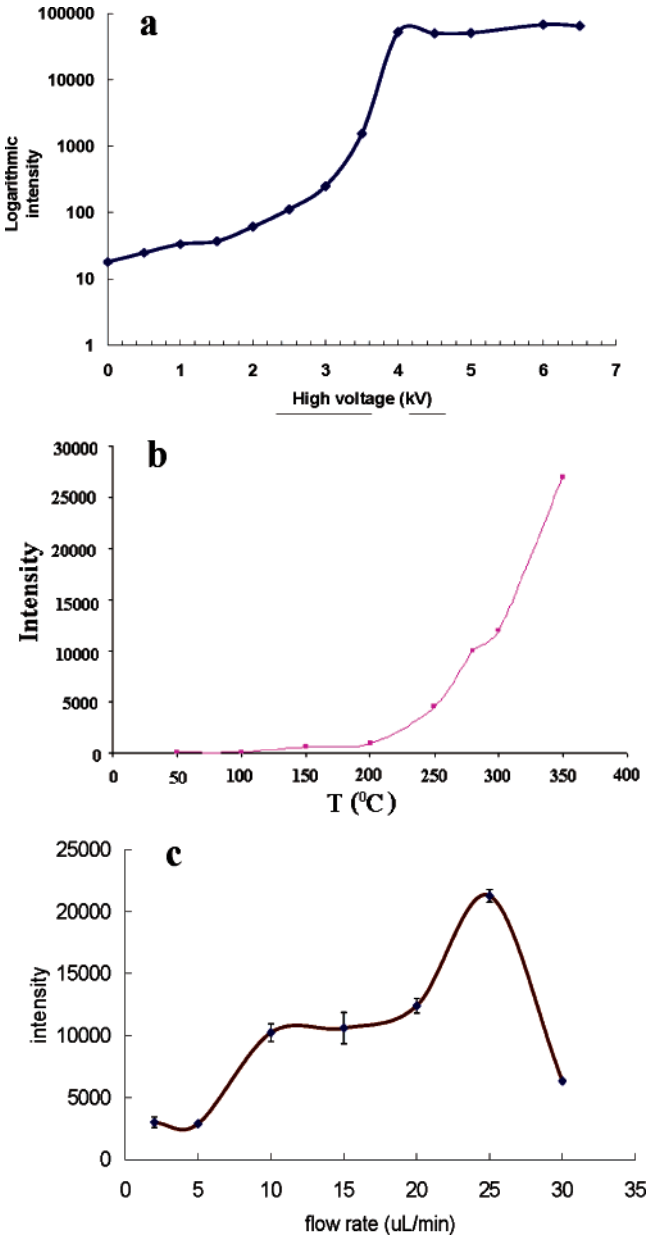

Figure 2. Effect of (a) electrospray voltage, (b) temperature of heated capillary, and (c) solvent infusion rate on the signal intensity of Claritin tablets in DESI.

charge transfer[43] at the surface to produce gas-phase ions of the analyte. Further discussion of the DESI mechanism occurs elsewhere,[35,44,45] but much remains to be done in order to elucidate it fully.

The effect of the heated capillary temperature on the signal intensity is shown in Figure 2b. The increased signal at higher temperatures may be due to more efficient desolvation. Higher temperatures also increase the chance of thermal degradation and gas-phase ion fragmentation; this occurs above 200 °C in the case of loratadine to yield the ions at m/z 337 and 339, by loss of neutral ethanol. The effect of solvent infusion rate on signal intensity was also examined experimentally As shown in Figure 2c, signal increases with infusion rate, going through a maximum and then rapidly decreasing. Increasing infusion rate increases both primary ion current and average droplet size, and this leads to higher analyte ion currents. The peak ratios were found to display only small changes with variations in total ion current for all the parameters reported here.

Using the 3D moving stage, other solid pharmaceutical samples containing a single active ingredient such as folic acid and the antiinflammatory agent acetaminophen were also investigated (Figure 3). Tandem MS was also applied in these cases, and fragmentation characteristics of the active ingredients were observed when the ionized molecules were mass-selected and subjected to collision-induced dissociation (insets in Figure 3). In the case of folic acid (Figure 3a), the only active ingredient present in the tablet, the protonated molecule gives an abundant signal at m/z 442, while the sodiated molecule, $(M + Na)^+$, is observed at m/z 464. Other peaks at m/z 313 and 295 may be due to elimination of the glutaric acid side chain as a radical by N−C cleavage and by dehydration of this product. In the case of acetaminophen tablets (Figure 3b), the protonated active ingredient is observed at m/z 152 while the main fragment at m/z 110 corresponds to loss of ketene. This is observed in both the MS and MS/MS spectrum of acetaminophen and is consistent with results observed by Zhou and Gilpin.[46] Considerable fragmentation is evident in the spectra of these compounds, associated with the high heated capillary temperature (275 °C) used to eliminate memory effects. Temperature effects on fragmentation vary with the compound, some like folic acid fragment easily in single-stage DESI-MS and acetaminophen (the latter in both the positive and negative ion modes) and some only in the MS/MS spectrum, like aspirin. The temperature was fixed for the entire set of experiments; hence, different compounds showed different degrees of fragmentation.

The inert matrix can also influence the appearance of the mass spectrum. For example, aspirin and melatonin tablets contain sodium salts in the matrix, and formation of the sodiated ions $(M + Na^+)$ at m/z 203 and 255 is favored over the protonated molecules $(M + H^+)$ (Figure 4). In the aspirin tablet (Figure 4a), the protonated form of acetylsalicylic acid is absent but the sodiated ion at m/z 203 is the base peak. CID of the m/z 203 peak yields the peak at m/z 161, a characteristic elimination of ketene. The peak at m/z 143 is the result of a further loss of water, and the minor peak at m/z 175 is due to a loss of 28, probably CO from the ion m/z 203.

In tablets containing more than one active ingredient, multiply protonated molecule ions are observed. In the case of melatonin tablets (Figure 4b), the data acquired for a single tablet using a 3D moving stage show ions due to the secondary ingredients vitamin B6 and dextrose at m/z 169 and 199. Other peaks such as m/z 241, 282, and 361 probably arise from additives, which include mineral oils, cellulose, Polysorbate 80, and dextrin. These matrix signals were not characterized further. Sodiated melatonin gives rise to the peak at m/z 255. The MS/MS spectrum shows fragment ions with m/z 212 corresponding to a loss of the acetyl group and the fragment of m/z 196, which is due to a loss of

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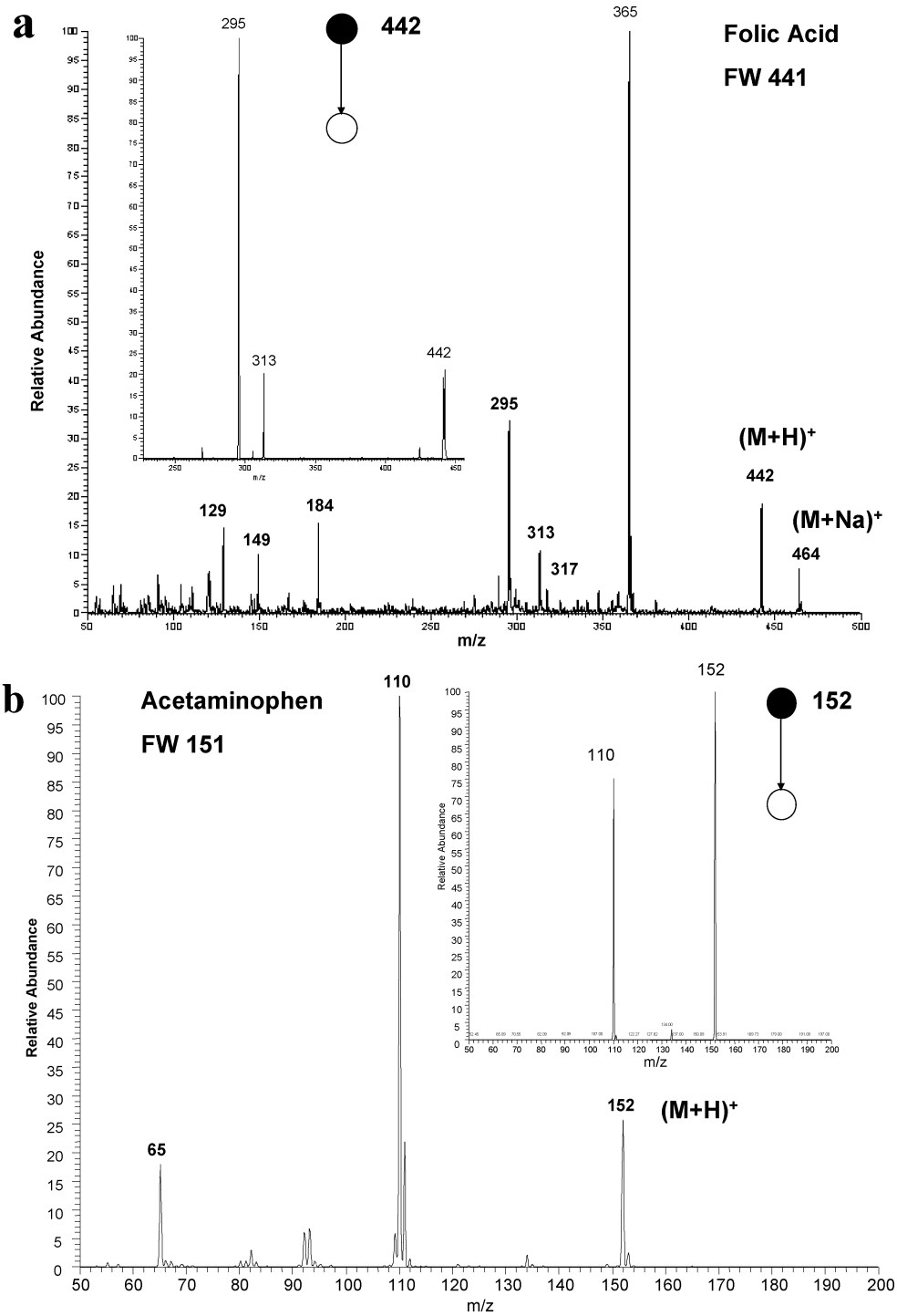

Figure 3. (a) DESI-MS and MS/MS spectra of *m*/*z* 442 (inset) folic acid in folic acid tablets and (b) DESI-MS and MS/MS spectra of *m*/*z* 152 (inset) acetaminophen in acetaminophen tablets using methanol/water (1:1) as spray solvent in the positive ion detection mode.

acetamide (Figure 4). A general comment that can be made at this point is that matrix effects are likely in DESI in the analysis of trace analytes present in complex matrices. This is not a complication in the samples examined in this study, which are successful because the active ingredients (melatonin in Figure 4) have reasonably high concentrations. The strong signals (Figure 4b) show the capability of DESI to directly detect the active ingredient in the complex melatonin tablet matrix. While DESI is a good method for qualitative analysis of components from a mixture, being relatively insensitive to matrix in the cases reported on here, its applications as a quantitative tool is likely to be more limited.

Representative drugs formulated as combinations of multiple active ingredients including Excedrin and Centrum tablets were studied (Figure 5). In the positive ion mode, all three active ingredients of Excedrin, acetaminophen, acetylsalicylic acid, and caffeine, are evident, as indicated in Figure 5a. The corresponding signals due to the protonated molecules are expected at *m*/*z* 152, 181, and 195. Acetylsalicylic acid did not appear as the protonated molecule, but the sodium adduct gives the most abundant peak

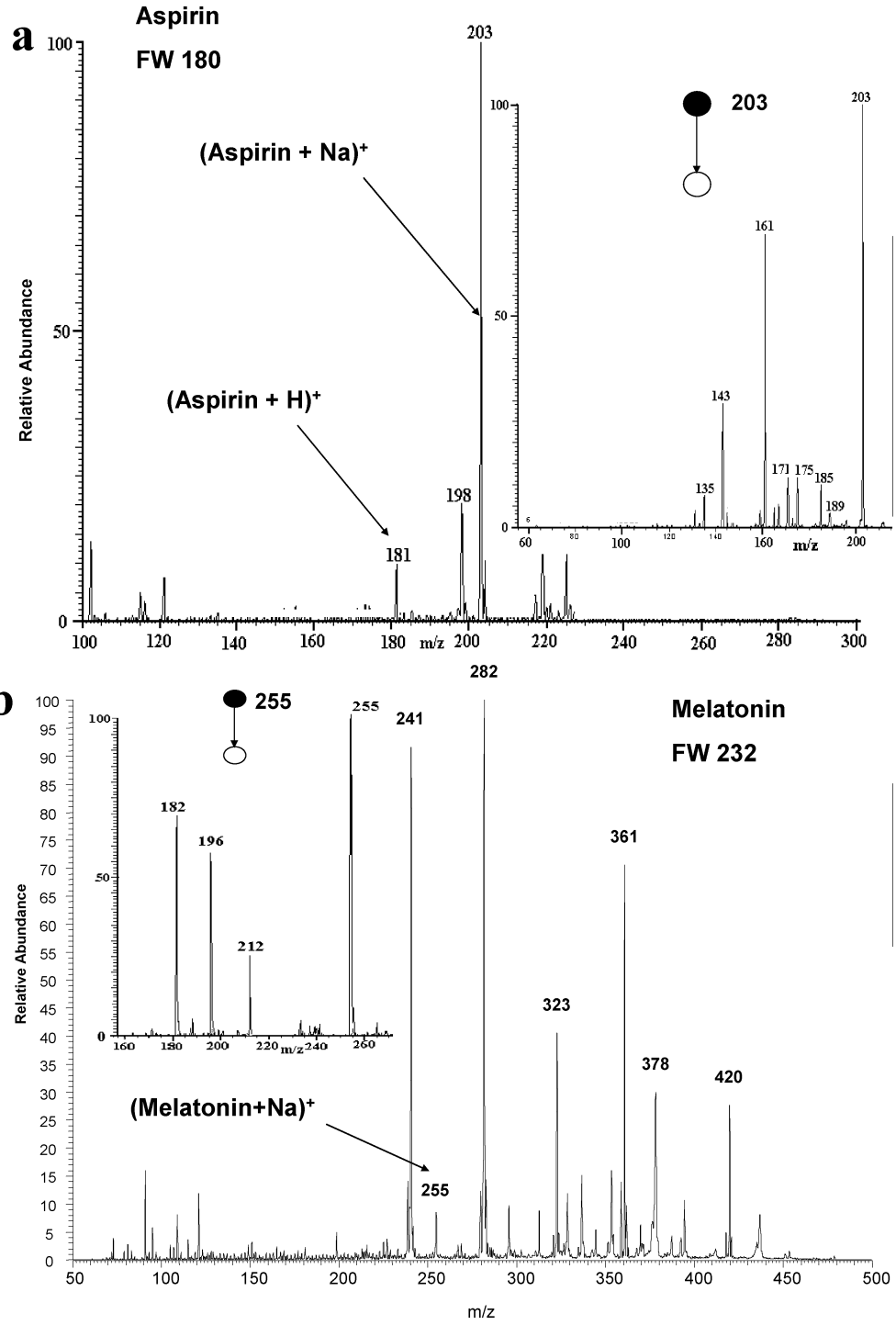

Figure 4. (a) DESI-MS and MS/MS spectra of aspirin (inset) in aspirin tablets showing cluster of aspirin *m/z* 203 with sodium as more abundant than the protonated molecule. (b) DESI-MS and MS/MS spectra (inset) of melatonin in melatonin tablets (3 mg of melatonin/tablet) using methanol/water (1:1) as spray solvent in positive ion detection mode.

in the spectrum of Excedrin. The two expected protonated forms of the active molecules appeared at *m/z* 152 and 195 (Figure 5a). and their identities have been confirmed by tandem mass spectrometry. The MS/MS spectrum of caffeine shows loss of CO to give a peak at *m/z* 167 while the ion of *m/z* 138 corresponds to loss of a neutral of mass 57 in agreement with results obtained by Toumi et al.[47] When recorded from the Excedrin tablets, acetaminophen also produced a sodium adduct in the MS spectrum (Figure 5a) at *m/z* 174, the main fragments of this species being recorded in the MS/MS spectrum listed in Table 1. Another example of the simultaneous detection of multiple active ingredients is provided by Centrum tablets. Using DESI-MS/MS, almost all of the vitamins in Centrum tablets can be detected in a single scan, as shown in Figure 5b. Note that the amounts of lutein and lycopene in a tablet are only 0.15 and 0.2

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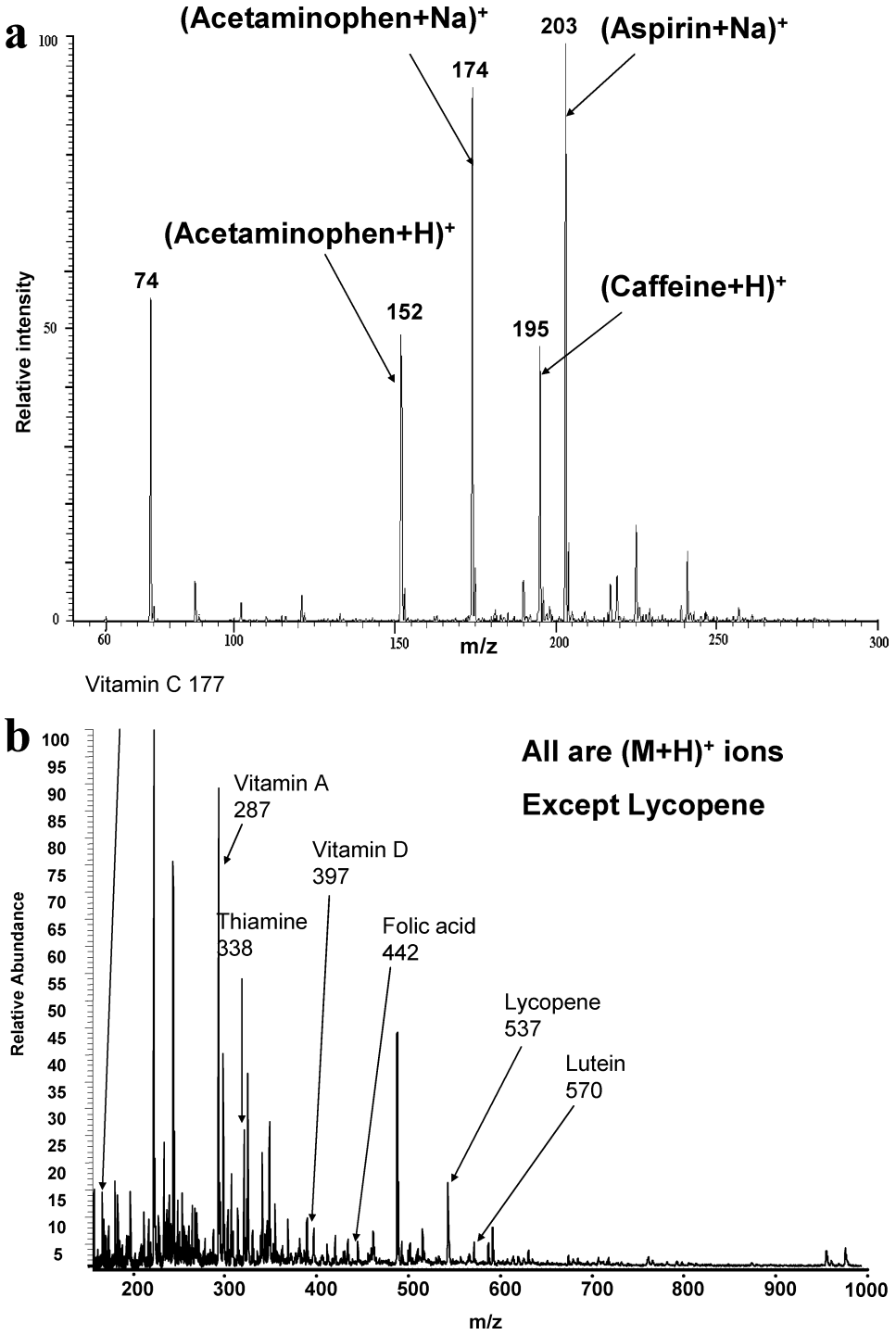

Figure 5. (a) DESI-MS of multiple active ingredients (aspirin, acetaminophen, and caffeine) in Excedrin tablets detected simultaneously by DESI-MS using methanol/water (1:1) as spray solvent in positive ion detection mode, where the sodium cluster of aspirin (*m*/*z* 203) and acetaminophen (*m*/*z* 174) is more abundant than the protonated molecule. (b) DESI-MS spectra of multiple components in Centrum tablets. Lycopene and lutein are only present at levels of 0.2 and 0.15 mg/g, respectively. This spectrum shows detection of minor components present at 0.01% (w/w) range in pharmaceutical samples.

mg, respectively. It is interesting that lycopene, in the Centrum tablets is detected both as the radical cation ($M^{\bullet+}$) and the protonated molecule $(M + H)^+$, a feature ascribed to its relatively low proton affinity.[48] This assignment is confirmed by the fact that there are usually two adjacent peaks, corresponding to the radical molecular cation and to the protonated molecule. Their relative abundance ratio can be changed by varying source conditions. The MS/MS data of these main active components were also recorded, and the main fragments are included in Table 1.

Table 1 summarizes all the tablets analyzed, their molecular weights, the amounts of active ingredients present as weight percent, the corresponding form of molecular ion observed in

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Table 1. DESI-MS/MS Data for Main Ingredients in the Tablets Investigated

tablets	ingredients	molecular weight	active ingredient (wt %)	molecular ion (m/z) ($M + H^+$)	main fragments MS/MS
Centrum	vitamin A	286	1225 IU	287	305, 263, 245, 232, 215
	β-carotene	536	3500 IU	537	495, 478, 419
	vitamin C	176	60 mg (4.0)	177	159, 149, 116, 101
	vitamin D	396	200 IU	397	379, 373, 355, 341, 239
	vitamin E	431	60 IU	432	407, 390, 362, 344, 275
	thiamine	301	4.5 mg (0.30)	302	278, 260, 242
	riboflavin	338	5.1 mg (0.34)	339	315, 397, 275, 257, 181
	folic acid	441	0.4 mg (0.03)	442	425, 401, 383, 322, 316, 239, 221
	biotin	1016	0.4 mg (0.03)	1017	955, 803, 544, 535, 355, 326
	vitamin B6	169	6 mg (0.40)	170	188, 152
	pantothenic acid	396	10 mg (0.67)	397	355, 337, 309, 239
	lycopene	537	0.2 mg (0.013)	537	479, 420, 496
	lutein	569	0.15 mg (0.01)	570	527, 506, 497, 439, 376, 294, 175
melatonin	melatonin	232	3 mg (0.6)	255 ($M + Na^+$)	212, 196, 182
	vitamin B6	169	10 mg (2.0)	170	188, 152
	dextrose	198	n/a	199	181, 168
aspirin	acetylsalicylic acid	180	325 mg (81)	181	138, 120, 92
folic acid	folic acid	441	5 mg (5)	442	425, 401, 383, 322, 316, 239
acetaminophen	acetaminophen	151	500 mg (80)	152	110, 65
Excedrin	acetaminophen	151	250 mg (36)	152	110, 65
	acetylsalicylic acid	180	250 mg (36)	181	138, 120, 92
	caffeine	194	65 mg (9.2)	195	167,138,95

Table 2. DESI-MS and MS/MS Data for Ointments and Liquid Solutions

sample phase	ingredients	molecular weight	molecular ion (m/z)	main fragments
ointment	ketoconazole	531.4	531.4 **(M^+)**	489, 463, 255, 244, 186
	1-(2-chlorotrityl)imidazole	344.5	345.6 **(M^+)**	310, 277, 266, 230, 130
solution	alanine in water	89	90 **($M + H^+$)**	72
	1,2,3-propanetriol in eye drops	92	93 **($M + H^+$)**	75, 57
	Zephiran Chloride in eye drops	424.15	425.3 **($M + H^+$)**	389, 301, 279, 256

DESI, and the key fragments observed in the MS/MS spectra. From this study, it is clear that DESI is useful for detection of multiple active components in solid pharmaceutical samples such as tablets using the positive ion detection mode.

Solid Tablets in the Negative Ion Mode. The use of DESI in the negative ion mode is motivated by the fact that negative ionization is more likely to yield selective and sensitive detection of those compounds that are easily deprotonated or have high electron affinities such as phenols and quinones. Many pharmaceutical agents fall into this class. Tablets of aspirin and acetaminophen were examined to demonstrate this useful feature of DESI for the detection of active ingredients. In the case of aspirin (Figure 6a) the deprotonated ion of acetylsalicylic acid (m/z 179) gives an abundant signal in the DESI spectrum obtained by using the 3D moving stage for single-tablet analysis. The MS/MS spectrum shows a peak at m/z 137 (salicylic acid) produced by loss of ketene during CID of the deprotonated molecule while m/z 151 is due to loss of CO from this same ion. The DESI mass spectrum of acetaminophen (Figure 6b) shows an abundant peak at m/z 150, the deprotonated molecular ion, while CID of this ion shows a prominent signal at m/z 107 due to loss of the acetyl group.

The negative ion mode was also used to examine pharmaceutical samples with multiple active ingredients, such as Excedrin tablets, previously examined in the positive ion mode. Abundant peaks at m/z 150 and 179 appeared as expected, corresponding to the deprotonated ions of acetaminophen and acetylsalicylic acid. Both assignments were confirmed by tandem mass spectrometry (Figure 6c). Caffeine is not seen in the spectrum, since it is unlikely to be deprotonated in acidic or neutral solvents[49] or by the ions generated from the methanol/water spray solvent. Therefore, the negative ion data are useful to complement the positive ion data in DESI since they provide greater signals for some compounds, such as acetylsalicylic acid. The choice of ionization mode depends on the chemical and physical properties of the analyte of interest as it does in other mass spectrometry experiments.

Ointments. Two ointments, clotrimazole and ketoconazole, containing 1-[(2-chlorophenyl)diphenyl-methyl]-1*H*-imidazole and 1-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethanone, both at 1% levels as active components, were chosen to be examined by DESI. A drop of cream (~10 mg) was spread over the surface of the sample holder (attached to the 3D moving stage), and the DESI mass spectrum was recorded from the surface of the ointment. The sample holder was attached to the 3D moving stage by a two-sided copper tape. As expected, given the relatively large amounts of active ingredient involved, signals due to protonated 1-(2-chlorotrityl)imidazole and ketoconazole are easily seen and the MS/MS data (Table 2) confirmed the assignments. These

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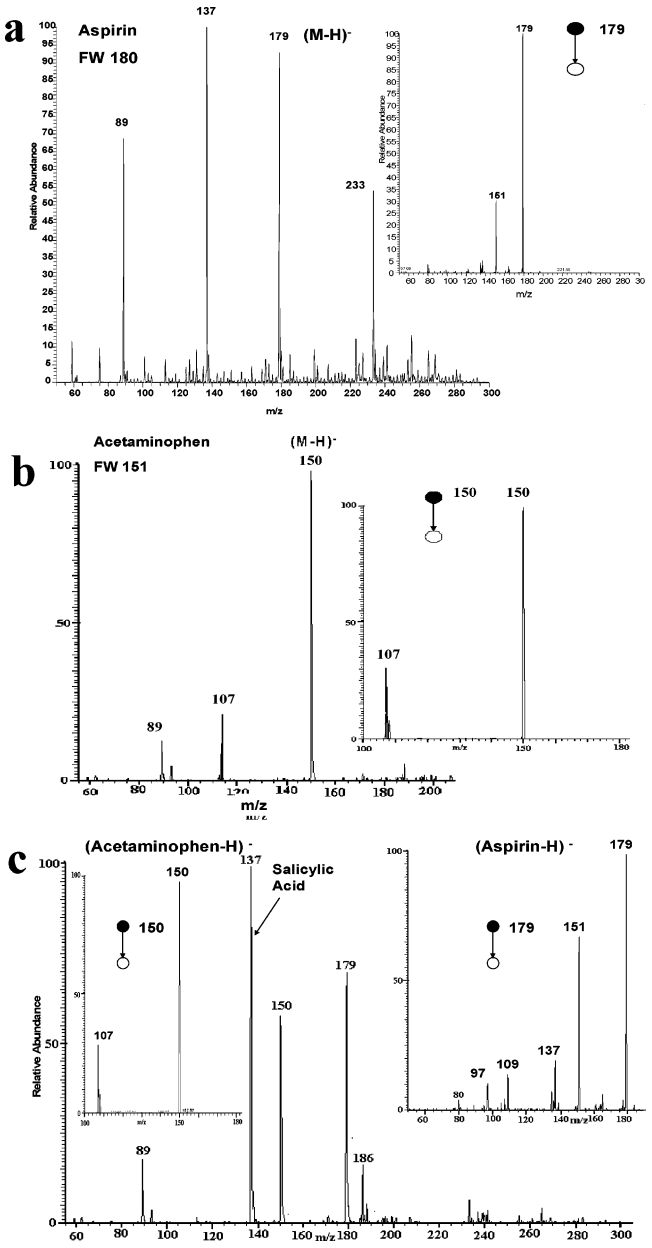


Figure 6. (a) DESI-MS and MS/MS spectra of aspirin (inset) in aspirin tablets using methanol/water (1:1) as spray solvent in the negative ion detection mode to yield deprotonated molecule of aspirin (*m*/*z* 179) and provide better sensitivity and a higher quality DESI spectrum than in the positive ion mode. (b) DESI-MS and MS/MS spectra of acetaminophen (*m*/*z* 150) in acetaminophen tablets in negative ion detection mode using methanol/water (1:1) as spray solvent. (c) DESI-MS and MS/MS spectra of aspirin and acetaminophen (insets) in Excedrin tablets in negative ion detection mode using methanol/water (1:1) as the spray solvent.

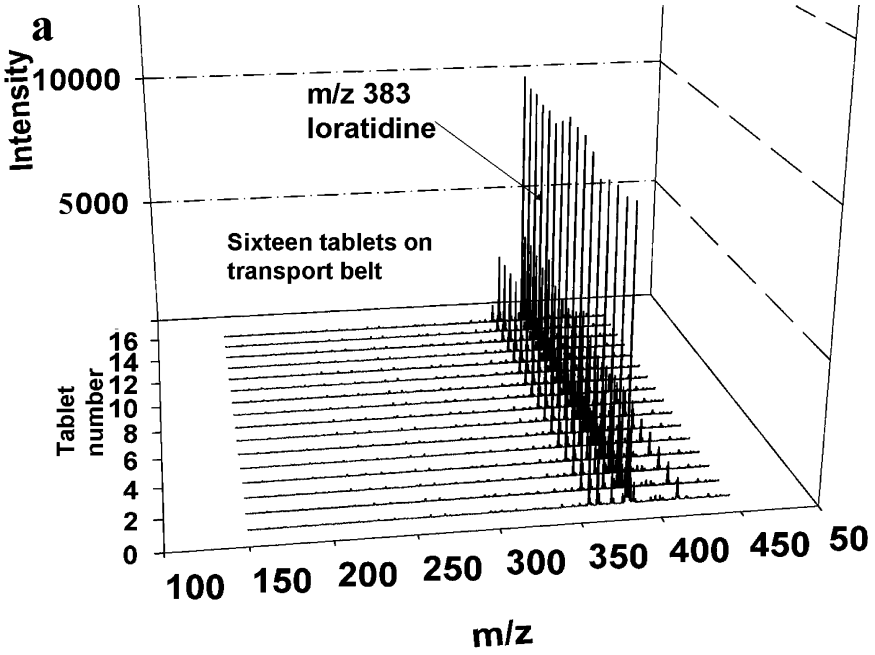
experimental data show that DESI-MS has the advantage of allowing direct monitoring of samples in ointments, an experiment that usually requires sample extraction and separation prior to mass spectrometry.

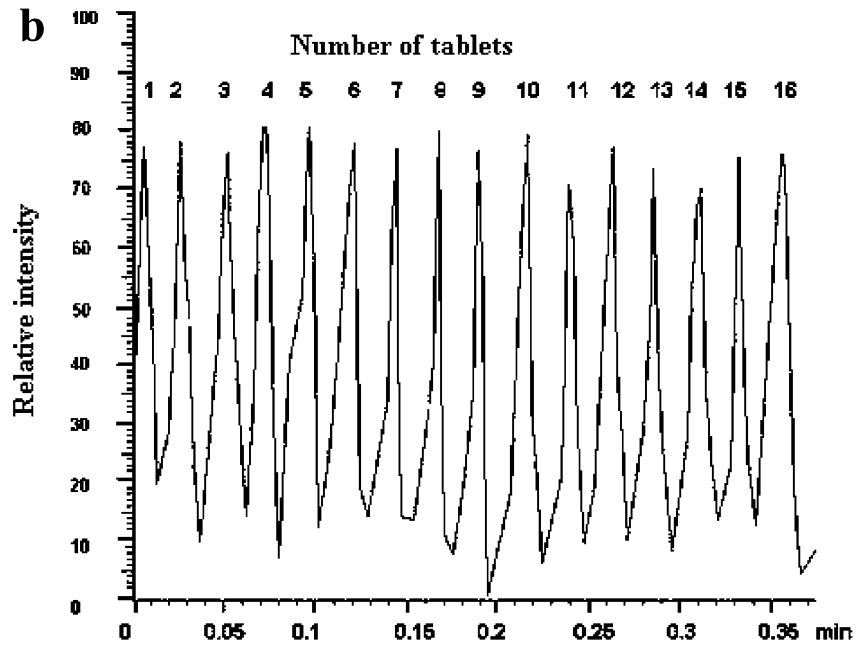
Liquid Pharmaceutical Preparations. Liquid-phase pharmaceutical samples are also used widely, an example being eye drop solutions. Bausch & Lomb eye drops, containing 1,2,3-propanetriol as active ingredient and Zephiran Chloride (alkyldimethylbenzylammonium chloride) as a secondary component, were investigated by DESI. A sample of 2 drops (~10 μL) of this liquid solution was added directly to the surface of filter paper, which had previously been attached to the surface of the 3D moving stage using two-sided copper adhesive tape, and DESI mass spectra were recorded on the dried surface. Peaks due to protonated 1,2,3-propanetriol (*m*/*z* 93) and Zephiran Chloride (*m*/*z* 425) are evident in the spectrum. The peak assignments are confirmed by the MS/MS spectra of protonated Zephiran Chloride and protonated 1,2,3-propanetriol, the main fragments of which are included in Table 2.

High-Throughput Analyses of Pharmaceutical Preparations. High-throughput experiments were carried out by using Claritin and acetaminophen tablets as samples, employing the moving belt system described in the Experimental Section. The sample transport system was successful in placing sample at the appropriate positions with respect to the DESI source and allowing good-quality mass spectra to be recorded despite its rudimentary nature. Flow rate responses were tested for both the static and high-throughput experiments and were not greatly different. By selecting the belt speed, high-throughput analysis of Claritin tablets was performed at different sampling rates ranging from 0.1 to 2.67 samples/s. A series of 16 mass spectra for 16 Claritin tablets acquired at a speed of 0.76 samples/s is shown in Figure 7a. All the spectra have almost the same relative ion intensities for the peak *m*/*z* 383. The relative standard deviation (RSD) of ion abundances of the base peak *m*/*z* 383 for all 16 tablets is calculated to be 4.8% (Table 3). However, the relative standard deviation of the ratio of intensity of *m*/*z* 383 to fragment *m*/*z* 311 for the 16 tablets is just 2.8%. Figure 7b shows the total ion signal for the same 16 Claritin tablets in the same experiment. Both panels a and b of Figure 7 demonstrate that the 16 tablets are easily resolved and characterized by full mass spectra at an operating frequency of 0.76 samples/s. Other typical data recorded at 2.67 samples/s operating frequency are shown in Figure 7c, for three sets of 16 tablets. All 16 tablets remain resolved at this analysis speed, but the RSD of the ion abundance of the base peak *m*/*z* 383 in each set of tablets increased to 7.8% (Table 3). The increasing RSD of the signal intensity with increasing sampling frequency is associated with the smaller data acquisition time and also to a less reproducible sampling position, since the moving belt also makes more lateral movements at higher speed. A more stable moving belt system is likely to allow considerably higher sample throughput than the 1000 samples that can be analyzed in 5 min at present.

MS/MS data could also be recorded in a high-throughput fashion, and this was demonstrated using acetaminophen. By monitoring of product ion of *m*/*z* 110 generated by dissociation of the parent ion, protonated acetaminophen (*m*/*z* 152), high-throughput MS/MS data were generated. A typical result is shown in Figure 7d in which the sampling rate is 1.53 tablets/s. Each of the 13 tablets examined yielded the fragment at *m*/*z* 110, although these MS/MS data show poorer RSD (~20%) when compared with high-throughput MS experiments (~8%).

Sample Carryover in High-Throughput Experiments. A factor that limits performance at high sampling speeds is sample carryover, a common effect in mass spectrometry. In this study, carryover effects were quantified by measuring the time required


a
Intensity
10000
5000
m/z 383
loratidine
Sixteen tablets on
transport belt
Tablet
number
16
14
12
10
8
6
4
2
0
100 150 200 250 300 350 400 450 50
m/z


b
Number of tablets
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Relative intensity
100
90
80
70
60
50
40
30
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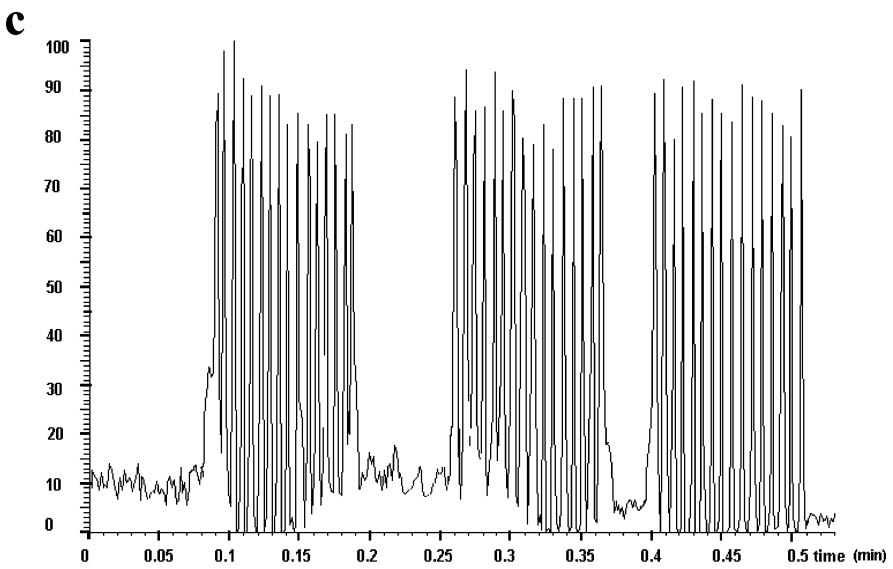
10
0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 min


c
100
90
80
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60
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0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 time (min)

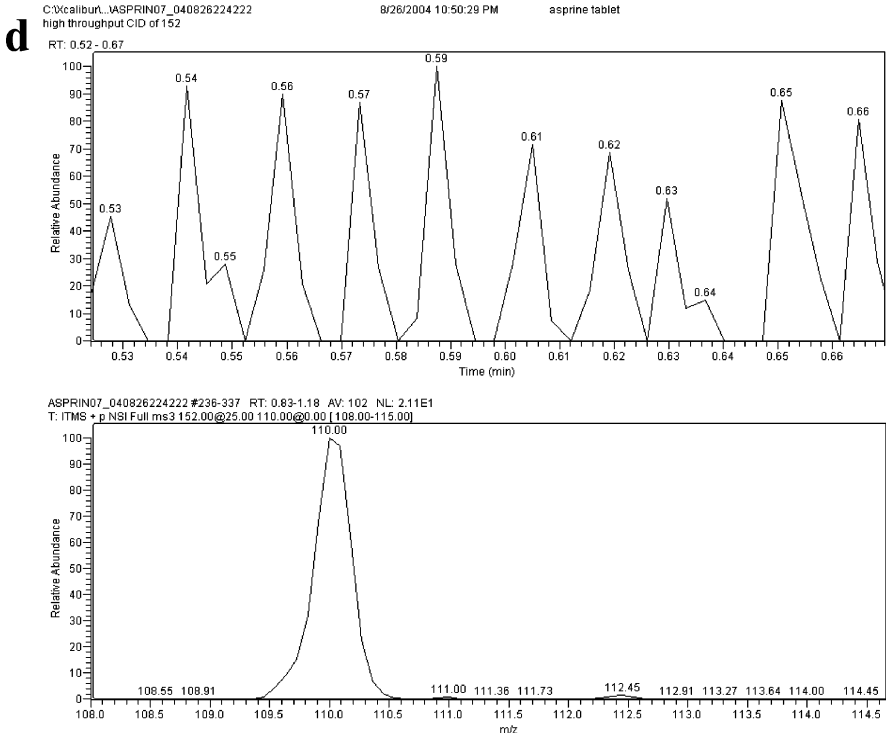

Figure 7. (a) Individual mass spectra of the 16 Claritin tablets on a moving belt at a speed of 0.76 sample/s. The main peak observed is at *m*/*z* 383 and the chloride signature is also observed at 385. All the peaks at *m*/*z* 383 for all tablets have almost identical intensities with the standard deviation being ~2%. (b) Ion chromatogram of high-throughput DESI (0.76 sample/s). By monitoring of ions at *m*/*z* 383, 16 Claritin tablets were investigated using the moving belt sample transportation system. (c) Ion chromatogram of high-throughput DESI (2. 67 samples/s). By monitoring of ions at *m*/*z* 383, three sets of 16 Claritin tablets were investigated using the moving belt as sample transportation system. (d) Ion chromatogram and mass spectrum of high-throughput DESI-MS/MS analysis of acetaminophen in acetaminophen tablets High-throughput DESI-MS/MS was carried out by monitoring characteristic fragments such as *m*/*z* 110 for the parent ions of protonated acetaminophen (*m*/*z* 152).

Table 3. Relative Standard Deviation (%) of Peak Intensities and Intensity Ratios in High-Throughput DESI Analysis of Claritin Tablets

sampling frequency (samples/s)	TIC[a]	I_{383}[b]	I_{383}/I_{385}[c]	I_{383}/I_{311}[d]
0.76	4.2	4.8	2.2	2.8
2.76	8.5	7.8	3.1	3.5

[a] Relative standard deviation of the total ion current for 16 tablets. [b] Relative standard deviation of the base peak intensity *m*/*z* 383 for 16 tablets. [c] Relative standard deviation of the ratio of intensity of *m*/*z* 383−385 for 16 tablets. [d] Relative standard deviation of the ratio of intensity of *m*/*z* 383−311 for 16 tablets.

for the signal intensity to drop from 100 to 10%. This was found to be less than 0.1 s for most tablets. In the high-throughput operation mode, most tablets can be separated by a valley of 1−10% of the peak height (Figure 7b) and considered to be resolved. Several experimental parameters having a strong influence on signal decay times were identified; these include spray parameters (solvent infusion rate and gas inlet pressure), geometric parameters (impact and collection angles, spray tip-to-sample and sample-to-heated capillary distances), and heated capillary temperature. The main factor limiting the observed resolution is not a consequence of the fast mass spectrometer scan time; instead it is due to the fall time of the signal, which is slow because of analyte memory effects in the atmospheric interface. We have tried to minimize this effect by keeping the temperature of the LTQ capillary high (275 °C). However, at very high temperatures, fragmentation takes place and some signal intensity is lost for the molecular ion, thereby reducing sensitivity. It is important to note that the optimum conditions for low carryover are not the same as those that are optimal for high sensitivity and a balance has to be maintained between these two conditions. If the DESI mechanism responsible for the carryover process involves a droplet−surface interaction, the problem can be reduced by avoiding the introduction of droplets into the heated capillary. Optimally, the DESI ion source should produce droplets that are large enough to hit the surface of tablets, but the droplets leaving the surface should be sufficiently small to undergo complete evaporation before entering the heated capillary. Parameters determining droplet size (solvent flow rate, gas inlet pressure) are thus optimum (to avoid carryover) when the droplets are just large enough to cause ionization. The data clearly show that the shortest signal fall times are observed at the lowest infusion rates and highest gas pressure that still gave measurable ion currents.

Semiquantitiative Measurements in the High-Throughput Mode. In the course of high-throughput analysis, the overall sample-to-sample reproducibility of the total ion current and base peak intensity can be quite low but the relative abundance of ions in the mass spectrum can show considerably better reproducibility, even at 2.76 samples/s (see Table 3). This better precision

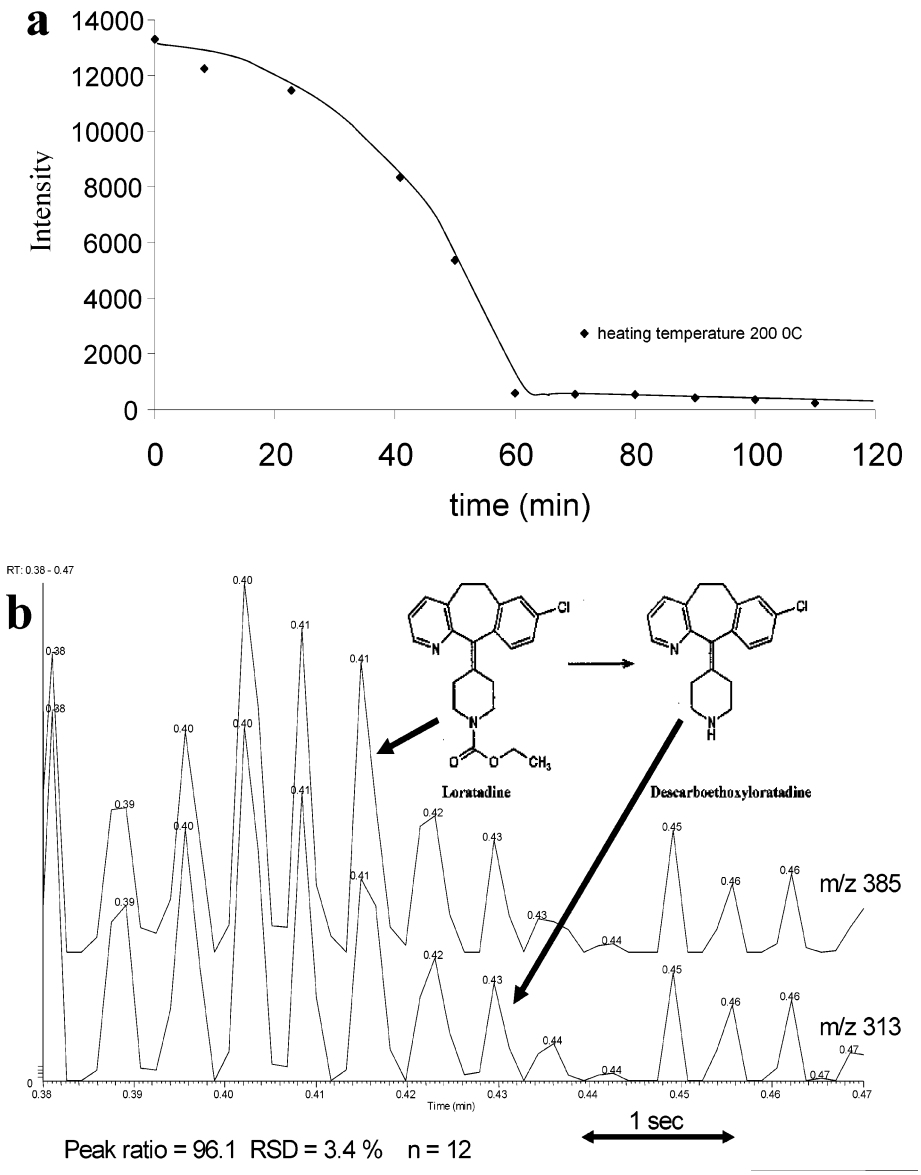

Figure 8. (a) Thermal degradation curve for Claritin tablets heated at 200 °C while monitoring *m*/*z* 383, the main active component in Claritin tablets. (b) Single ion currents corresponding to loratadine and its descarboethoxy degradation product. Claritin tablets were heated at 200 °C for 30 min, to cause thermal dissociation.

includes active ingredient/impurity ratios as well as ratios of peaks due to one or more active ingredients. To model a process failure in a production line, some Claritin tablets were heated prior to analysis for different amounts of time to different temperatures, the highest being 200 °C, at which point the sample had visibly degraded. Degradation curves for the Claritin tablets are shown in Figure 8a. The treated samples show considerably lower signal intensities for loratadine and higher intensities for the decomposition products at m/z 381 and 311 (Figure 8b). Both the drop in the loratadine intensity and the increase in the decomposition product intensities were proportional to the heating time. These initial results suggest that DESI-MS can be used for high-throughput impurity profiling and process control for complex systems in the pharmaceutical industry at sampling rates as high as 2−3 samples/s.

CONCLUSIONS

High-throughput analysis of pharmaceuticals by DESI demonstrates the capabilities of this method for quality assurance. This is clearly just one example of a high-throughput application of this new ionization method. Tablet analysis itself can be used for forensic purposes too, for instance, for the analysis of counterfeit drugs. The fact that high-throughput DESI experiments apply to pharmaceutical preparations in a variety of physical states should be considered in conjunction with an earlier report[35] where it has been be applied to the analysis of body fluids such as full blood, serum, or urine in the form of dried spots for various purposes including pharmacokinetics, diagnostics, and detection of drugs of abuse. These capabilities indicate that the method has potential value in a variety of pharmaceutical and biomedical applications, as also is indicated by recent conference papers.[44,50,51]

The most advantageous feature of DESI is that arbitrarily chosen objects can be sampled in a minimally invasive way. As shown in the present study, the sampling rate, sensitivity, and cross-contamination between samples are all acceptable in achieving high throughput and high specificity with adequate sensitivity. The specificity of detection of the active ingredients, even when present in small amounts in the presence of matrix, follows from the fact that tandem mass spectrometric experiments can also be done in the same high-throughput fashion.

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