Direct monitoring of drug degradation by easy ambient sonic-spray ionization mass spectrometry: the case of enalapril

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Using enalapril maleate as a test case, the ability of ambient mass spectrometry, namely, via easy ambient sonic-spray ionization mass spectrometry (EASI-MS), to perform direct monitoring of drug degradation has been tested. Two manufacturing processes were investigated (direct compression and wet granulation), and the formation of degradation products was measured via both EASI-MS and high-performance liquid chromatography with ultraviolet detection for a total period of 18 months. Both techniques provide comparable results, which indicate that direct analysis by ambient mass spectrometric techniques presents a viable alternative for drug degradation monitoring with superior simplicity, throughput, and reliability (no sample manipulation), and comparable quantitative results. In terms of qualitative monitoring, the full mass spectra with intact species provided by EASI-MS allow for comprehensive monitoring of known and unknown (or unexpected) degradation products. Copyright © 2011 John Wiley & Sons, Ltd.

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INTRODUCTION

The stability of a pharmaceutical formulation is a major factor affecting the quality of drug products and their efficacy.[1] Drug stability is mainly affected by the exposure of the product to environmental conditions such as temperature, humidity, and light. Its chemical composition and the physical-chemical properties of excipients and active ingredients and their relative quantities in the formulation as well as the manufacturing process and conditions of storage and transportation are also major factors influencing drug stability. Metabolites created in the human body are also crucial for overall efficacy and safety of a drug. In modern pharmaceutical drug discovery and development, it is of crucial importance to identify unknown compounds arising from impurities or degradation with the highest possible confidence because of their potential pharmacological effects on humans.[2,3]

Recently, a set of techniques known collectively as ambient ionization mass spectrometric techniques[4] have been developed and applied with success to the direct analysis of drug formulations.[5] We have also introduced an ambient ionization technique, namely, easy ambient sonic-spray ionization (EASI),[6,7] and tested it for direct drug analysis with superior simplicity and signal-to-noise ratios.[8] EASI produces a bipolar stream of charged droplets and is also attractive for being inherently free from electrical or discharge interferences and of greater simplicity because it requires no voltage and temperature or irradiation assistance and may incorporate Venturi pumping.[9] Herein we tested whether ambient ionization mass spectrometry, as exemplified by EASI, applied direct to the drug formulation without any sample preparation or pre-separation, would provide reliable monitoring of drug degradation as compared with the more conventional monitoring performed via high-performance liquid chromatography–ultraviolet (HPLC-UV) using drug extracts. Enalapril maleate, a drug that acts as an angiotensin-converting enzyme inhibitor, constitutes one of the major drugs used to treat essential and renovascular hypertension and congestive heart failure,[10] was used in commercial formulations as a test case. Two manufacturing processes were also compared.

EXPERIMENTAL

Chemicals

Formic acid, methanol, acetonitrile, and phosphoric acid were purchased from Sigma-Aldrich (São Paulo, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore, Billerica, MA, USA) purification unit.

Mass spectrometry

The EASI-MS experiments were performed in a mass spectrometer (LCMS-2010EV-Shimadzu Corp., Japan) equipped with a homemade EASI source described in detail elsewhere.[8] To produce
the sonic spray, an acidic solution of methanol (0.1 v%) at 20 μl min⁻¹ and N₂ (100 psi) was used. Experiments were performed in the positive ion mode.

High-performance liquid chromatography
The HPLC-UV is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The column temperature was maintained at 50 °C, and the flow rate was of 2 ml min⁻¹. The injection volume was 50 μl. The buffer solution was prepared as follows: 1.38 g of monobasic sodium phosphate was dissolved in approximately 800 ml of water, and the pH was adjusted to 2.2 with phosphoric acid, followed by dilution with water to 1000 ml, and mixing. The mobile phase was a mixture of the buffer solution and acetonitrile (75:25). Ten tablets were used to prepare approximately 0.2 mg ml⁻¹ aqueous solution of enalapril maleate. The buffer solution was added in a 2 : 1 ratio, and the mixture was sonicated for 15 min and shaken by mechanical means for 30 min, then diluted with the buffer solution to volume, shaken again, and sonicated for additional 15 min. The resulting mixture was filtered (0.45-μm filter). HPLC quantitation of enalaprilat and diketopiperazine (DKP) were performed using U. S. Pharmacopia (USP) standards (Figure 1).

RESULTS AND DISCUSSION
Drug degradation was followed for a total period of 18 months, and analysis was performed every 3 months. Figure 2 shows the EASI(+)-MS for a fresh enalapril tablet (20 mg of enalapril maleate in a 200 mg ± 5% total weight) as well as representative spectra for a tablet after 12 and 18 months during natural aging at controlled conditions (climatic chamber at 40 °C and 75% relative humidity). For the fresh tablet (Figure 2a), the active drug, namely, the protonated enalapril molecule [M + H]⁺ of m/z 377 as well as its sodiated molecule [M + Na]⁺ of m/z 399 are the two predominant ions. However, two known degradation products of enalapril (Scheme 1), namely, enalaprilat as the [M + H]⁺ ion of m/z 349 and DKP as [M + Na]⁺ of m/z 381 are also detected for the fresh formulation, indicating degradation by hydrolysis and dehydration during manufacturing by the wet granulation process. Lactose, the major excipient, is also detected as [M + Na]⁺ of m/z 365, which is also beneficial for the monitoring of formulation and its stability. The other excipients (sodium bicarbonate, amide, silicon dioxide, magnesium stearate, and iron oxide) are not detected by EASI(+)-MS.

After a year inside a climatic chamber at 40 °C and 75% relative humidity, as expected, the abundance of the enalapril ions of m/z 377 and 399 decreases, with the subsequent increase of the relative abundances of the ions of m/z 381 and 349, whereas that from the lactose excipient (m/z 365) remained quite unaltered. After 18 months (Figure 2), however, degradation is quite severe, and the enalaprin ions of m/z 377 and 399 are much reduced in abundance whereas those for elaprilat (m/z 349) and DKP (m/z 381) are now dominant. Interesting, EASI-MS is also able at this point to detected further degradation products via the ions of m/z 252 and 331. Scheme 2 depicts two possible structures for these products of 251 and 330 Da.

Figure 3 shows the EASI-MS data for degradation monitoring for tablets manufactured by the direct compression process, via which the formulation is not subjected to high temperature or humidity. As compared with Figure 2, very similar spectra were obtained, but with some substantial differences in terms of
degradation extent. For the fresh sample, only the enalapril (m/z 377 and 399) and lactose (m/z 365) ions are detected with no signs of degradation. After 18 months of prolonged exposition to humidity and heat (climate chamber at 40°C and 75% relative humidity), the relative abundances of the enalapril ions decrease and those of the degradation products enalaprilat (m/z 349) and DKP (m/z 381) increase, but to lesser extents than those observed for the tablets manufactured by the wet granulation process. Note that the further degradation products detected via the ions of m/z 331 and 252 in Figure 2 (18 months) are not seen in Figure 3 (18 months).

Figure 4 shows a typical HPLC-UV chromatogram for an extract from a pool of 10 tablets of enalapril manufactured via the wet granulation process after 12 months of prolonged degradation. Note the peak for enalapril at 3.8 min and those for its widely known degradation products enalaprilat at 1.9 min and DKP at 11.7 min. The peak at approximately 1 min is due to maleic acid. Using USP standards and by constructing the corresponding calibration curves, quantitation was performed, and the results are summarized in Figure 5.

Figure 5 compares the degradation profiles obtained by both the HPLC-UV and the EASI-MS monitoring. Note that for HPLC-UV, quantitation was performed via classical procedures using USP standards for the degradation products. For EASI-MS, semi-quantitation is performed via direct comparison of relative abundances assuming equal ionization efficiencies. Although there is a small quantitative difference, qualitatively both profiles are quite similar indicating the usefulness of EASI-MS for direct monitoring of the degradation profile of this drug.

Figure 6 shows EASI-MS data for tablets manufactured using the wet granulation process and exposed to accelerated and severe degradation conditions, that is, (i) high acidic media as simulated by dropping a drop of an aqueous 1-N HCl solution directly on the top of the tablet and letting it dry at ambient conditions and (ii) high temperature as simulated by exposing the tablet to 100°C for 4 h. As compared with the prolonged degradation (Figures 2 and 3), EASI(+)-MS is able to detect two additional degradation products via the ions of m/z 313 and 303. Although secure characterization of these degradation products would require a more extensive structural investigation, Scheme 2 depicts two possible structures corresponding to [M–H2O–EtOH] of 312 Da and [M–CO–EtOH] of 312 Da.

![Scheme 1. Structures of Enalapril and two of its known degradation products.](image)

![Scheme 2. Putative structures for the Enalapril degradation products detected by EASI-MS.](image)

**Figure 3.** EASI-MS for enalapril manufactured by the direct granulation process at different periods of stability in a climatic chamber at 40°C and 75% relative humidity.
CONCLUSIONS

Using enalapril maleate as a test case, the ability of EASI-MS to monitor drug degradation directly from intact tablets has been demonstrated. As compared with classical HPLC-UV monitoring, EASI-MS has been shown to be able to provide similar qualitative and quantitative results with much superior speed and simplicity and with no need of sample preparation and pre-separation, and without inherent risks of contamination and artifacts associated with these procedures. Although the test case evaluated herein points to the viability of ambient ionization mass spectrometry to directly monitor drug degradation, the generality and robustness of the approach should be ideally tested with a comprehensive set of chemicals from different classes of drugs such as antibiotics, steroidal hormones, and vitamins. For most commercial drugs, however, a simple preliminary test could be easily performed by analyzing an extensively degraded tablet and by evaluating the ionization efficiencies of known degradation products. Because of ion suppression effects, it is also predictable that EASI-MS should work best for polar drugs forming polar degradation products. Fortunately this seems to be a common feature of many commercial drugs.

Figure 4. HPLC-UV chromatogram for a methanolic extract from a poll of 10 tablets of enalapril maleate produced by the wet granulation process after 12 months of prolonged degradation of a climatic chamber at 40 °C and 75% relative humidity.

Figure 5. Comparison of degradation profiles obtained by HPLC-UV (upper panel) and EASI-MS (lower panel) monitoring.
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REFERENCES


Figure 6. EASI-MS for enalapril manufactured by the wet granulation process and submitted to accelerated and drastic acid (concentrated HCl) and thermal (100 °C for 4 h) degradation.