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Short communication

Rapid identification and absence of drug tests for AG-013736 in 1 mg Axitinib tablets by ion mobility spectrometry and DART™ mass spectrometry

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ABSTRACT

Axitinib (AG-013736) is a potent investigational drug that has antitumor activity in patients with metastatic renal cell carcinoma and other types of cancers. In this study, ion mobility spectrometry and "direct analysis in real time" (DART™) mass spectrometry were used to rapidly identify AG-013736 in drug substance samples and 1 mg Axitinib tablets. The plasmagrams of the sample solutions exhibited a major peak with a reduced ion mobility that was within $\pm 0.0002\ cm^2\ V^{-1}\ s^{-1}$ of that for AG-013736 in an external reference standard solution. The DART ionization source was coupled with both a time-of-flight mass spectrometer and a lower-resolution ion trap mass spectrometer. Samples were analyzed by this technique in as little as 5 s with minimal to no sample preparation required. The isotopic masses of the protonated dimer ions of AG-013736 were used to identify AG-013736 in the active tablet. Both techniques were also used to develop low-level limit tests for rapidly verifying the presence or absence of AG-013736 in blinded clinical supplies of active and matching placebo tablets of Axitinib.

1. Introduction

Axitinib (AG-013736) is a potent and orally bioavailable drug that has exhibited promising antitumor activity, both as a single agent and in combination with other anticancer agents, during clinical trials in patients with metastatic renal cell cancer [1], advanced non-small-cell lung cancer [2], advanced thyroid cancer [3], and other types of tumors [4]. The chemical structure of AG-013736 is shown in Fig. 1.

Ion mobility spectrometry (IMS) and "direct analysis in real time" (DART™) mass spectrometry are rapid and sensitive techniques that have been used to detect trace levels of numerous compounds, including illicit drugs [5–8], explosives [7,8], and chemical warfare agents [9,10]. These techniques have also been used to distinguish between authentic and counterfeit drug products [5,11]. In addition, ion mobility spectrometry has been used to detect undeclared drugs in dietary supplements [12,13], and to differentiate 10 mg baclofen tablets from matching placebo tablets in blinded clinical supplies [14]. Unlike UV spectrometry, both of these techniques can detect analytes without chromaphores; in addition, columns and mobile phase solvents are not required as in HPLC.

IMS is a gas-phase electrophoretic technique that utilizes a weak electric field to separate ionized compounds based on factors such as their charge, mass, size, shape, and electrostatic interactions with the neutral molecules of the surrounding "drift gas," which is present at ambient pressure [15,16]. As they migrate through the drift gas, each ionic species quickly reaches a constant and characteristic "drift velocity" that is proportional to the applied electric field strength. The proportionality constant, corrected to standard temperature and pressure conditions, is known as the reduced mobility (K_0) of the ion. The time required for each ionized compound to reach the detector at the end of the drift tube is defined as its "drift time." A plot of ion intensity versus drift time is known as a plasmagram.

DART-MS, on the other hand, combines an ambient ionization source with a mass analyzer that separates the ions based on their mass-to-charge ratio (m/z) [7,17]. This ionization source generates a heated stream of excited-state gaseous atoms or molecules that are sprayed across an open-air gap toward the inlet of the mass spectrometer. The sample is placed within this stream and ionized before entering the mass spectrometer.

In this paper, we demonstrate how IMS and DART-MS can be used as orthogonal techniques to rapidly identify AG-013736 in AG-013736 drug substance samples and to verify the presence or absence of AG-013736 in 1 mg Axitinib and matching placebo tablets. These tablets were chosen because AG-013736 is present in the active tablets at only about 1.0 wt%, which can make absence of

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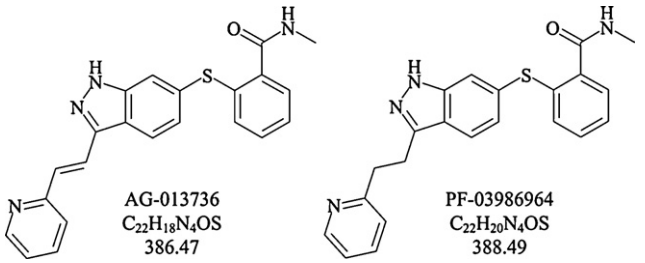

Fig. 1. Chemical structures of AG-013736 and PF-03986964.

drug tests challenging to develop. In addition, Axitinib and matching placebo tablets have been used in double-blind clinical studies, where the identity of each drug product batch must be confirmed prior to dosing patients.

2. Materials and methods

2.1. Samples and sample preparation

The reference standard lots of AG-013736 and PF-03986964 were greater than 99.0% and 97.5% pure, respectively. The 1 mg Axitinib and matching placebo tablets were manufactured using standard pharmaceutical excipients [18]. HPLC solvent grade methanol was obtained from J.T. Baker (Phillipsburg, NJ) or Sigma–Aldrich (St. Louis, MO). Ultra-high purity compressed helium (99.999%) was obtained from ABCO Welding Supply (Waterford, CT).

Unless otherwise stated, all solutions for ion mobility analysis were prepared at a concentration of ≈20 μg/mL in methanol and analyzed in duplicate. In addition, the average K_0 for each compound from these two analyses is reported. For identification testing, a 1 mg Axitinib tablet was crushed and transferred into an amber glass vial along with 50.0 mL of methanol. The vial was capped and shaken at about 200 oscillations per min for 30 min. The resulting suspension was then centrifuged to yield a clear supernatant, which was transferred into a 2-mL amber glass HPLC vial. A screw-on cap with a PTFE/silicone/PTFE septum was used to seal this vial. For absence of drug testing, the same procedure was followed except that 10 tablets were crushed and the combined powder was shaken in 10.0 mL of methanol.

For DART-MS analysis, the reference standard or drug substance powder was deposited onto a melting point capillary constructed of borosilicate glass. The film-coated Axitinib tablets were cut in half and a melting point capillary was used to scratch off some of the tablet core material for analysis. For absence of drug testing, 10 tablets were crushed and transferred into an amber glass vial. Approximately 10 μg of PF-03986964 dissolved in 10.0 mL of methanol was then added to this vial, and the sample was shaken and centrifuged as described above. The sample solution was then concentrated by allowing the methanol to evaporate to a final volume of about 1 mL. A melting point capillary was then dipped into the solution in order to obtain a sample for analysis.

2.2. Ion mobility spectrometry

The plasmagrams were obtained using a Model 400B IONSCAN®-LS ion mobility spectrometer that was equipped with a COBRA L/S autosampler and controlled using IM-Station v. 5.389 software from Smiths Detection Scientific (Danbury, CT). The spectrometer was operated in the positive ion mode using default control parameters and dried air as the drift gas. A 1-μL aliquot of the sample solution was deposited onto the PTFE filter in the sample introduction tray, the methanol was allowed to evaporate, and the sample was vaporized using desorber and inlet heater settings of 300 and 295 °C, respectively. The analyte was then ionized, presumably to the protonated molecular ion [15,16], by atmospheric pressure chemical ionization using a 15 mCi ^{63}Ni radioactive source. The two-dimensional plasmagram of the sample was obtained using a scan period of 25 ms, 50 co-added scans per segment, 80 segments per analysis, and an analysis time of 100 s. Nicotinamide was used as an internal calibrant to calculate the K_0 of the analyte as $K_0 = K_{0,nic}(t_{d,nic}/t_d)$, where $K_{0,nic}$ is the reduced ion mobility of nicotinamide (preset to 1.8600 $cm^2\,V^{-1}\,s^{-1}$) and $t_{d,nic}$ and t_d refer to the drift times of the nicotinamide and analyte ions, respectively.

2.3. DART mass spectrometry

The mass spectra were obtained by coupling a Model DART 100 ion source from IonSense, Inc. (Saugus, MA) to either a high-resolution time-of-flight (TOF) mass spectrometer or a lower-resolution ion trap instrument. In both cases, the ion source was operated in the positive ion mode. IonSense's DART v.1.55 software was used for instrument operation and data acquisition.

The individual samples were analyzed in as little as 5 s by manually positioning the melting point capillary between the gas flow exit of the ionization source and the orifice of the mass spectrometer while monitoring the total ion chromatogram. It is generally believed that the electronically excited helium atoms generated by the DART ion source react with ambient water vapor to produce hydronium ions that subsequently transfer a proton to the analyte [17,19]. Both the protonated molecular ion and the protonated dimer ion of AG-013736 were observed.

2.4. DART time-of-flight mass spectrometry

The DART-TOF mass spectra were obtained using the DART ionization source and a Model JMS-T100LC AccuTOF-DART™ mass spectrometer from JEOL USA, Inc. (Peabody, MA). A helium flow rate of 1.2 L/min, needle electrode voltage of 3.5 kV, and gas temperature of 300 °C were used for the DART ionization source. The mass spectrometer was controlled using typical parameters and JEOL's MassCenter Main v. 1.3.0.1000 software.

2.5. DART ion trap mass spectrometry

The DART source was also interfaced to an LCQ™ Deca XP ion trap mass spectrometer from Thermo Fisher Scientific (Waltham, MA) using a custom-built stage from IonSense. The ion trap instrument was operated in selective ion monitoring mode centered at m/z 387 and 389 for singly charged ions of AG-013736 and PF-03986964, respectively. An m/z of 773 and 777 were used for the corresponding dimer ions of these compounds. An m/z window of 20 was used in each case. Typical instrument parameters for the LCQ Deca XP were used for these analyses.

3. Results and discussion

3.1. Identification test by ion mobility spectrometry

The plasmagram of the AG-013736 reference standard is shown in Fig. 2. The K_0 values of AG-013736 and PF-03986964, which is a potential process-related impurity of AG-013736 [20], ranged from 0.9411 to 0.9413 and 1.0180 to 1.0182 $cm^2\,V^{-1}\,s^{-1}$, respectively, based on six injections of a 20-μg/mL solution of AG-013736 and a 1-μg/mL solution of PF-03986964. These compounds are well resolved (e.g., $R \approx 2.0$ for a mixture containing 10 μg/mL of each) [13], even though they have similar masses and chemical structures as shown in Fig. 1.

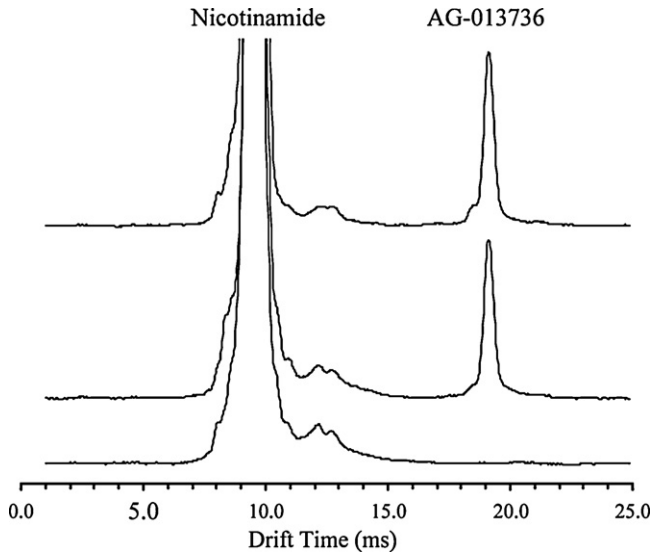

Fig. 2. Representative plasmagrams of the AG-013736 reference standard solution (top), a 1 mg Axitinib tablet preparation (middle), and a placebo tablet preparation (bottom).

The K_0 of AG-013736 was also independent of drug concentration over a wide range, even though the peak height response becomes nonlinear at concentrations greater than ≈6.0 μg/mL. For example, thirteen solutions were prepared between 0.6 and 19.3 μg/mL and the K_0 of AG-013736 ranged from 0.9404 to 0.9409 $cm^2\ V^{-1}\ s^{-1}$.

In addition, 20-μg/mL solutions of AG-013736 were analyzed on different days over a one-year period using two ion mobility spectrometers located in different laboratories within the same building, and the K_0 for AG-013736 was found to be reproducible—ranging from 0.9404 to 0.9412 $cm^2\ V^{-1}\ s^{-1}$.

This method was also used to identify AG-013736 in three drug substance lots prepared at a concentration of ≈3 μg/mL. The plasmagrams of the sample solutions exhibited a major peak with a K_0 value that was within ±0.0002 $cm^2\ V^{-1}\ s^{-1}$ of that for the AG-013736 peak in the plasmagram of the external reference standard solution.

The plasmagrams of the 1 mg Axitinib and placebo tablet preparations are compared to that of the AG-013736 reference standard solution in Fig. 2. The plasmagram of the active tablet solution also exhibited a major peak with a K_0 value that was within ±0.0002 $cm^2\ V^{-1}\ s^{-1}$ of that for AG-013736 in the reference standard solution. In addition, there are no excipient-related peaks that interfere with the identification of AG-013736.

This method was also used to correctly differentiate between active and matching placebo tablets that were submitted as blinded samples for analysis.

3.2. Absence of drug test by ion mobility spectrometry

Ion mobility spectrometry can also be used with more concentrated solutions to demonstrate the absence of drug in placebo tablet samples. For example, the plasmagrams in Fig. 3 show that this technique is sensitive enough to detect less than 0.1% of the total amount of AG-013736 that would be present in a composite sample of 10 active tablets. This level is the same as the ICH reporting threshold for degradation products in new drug products with maximum daily doses of 1 g or less [21]. Therefore, this method can be used as a limit test to demonstrate that AG-013736 is not present in these tablets above this threshold.

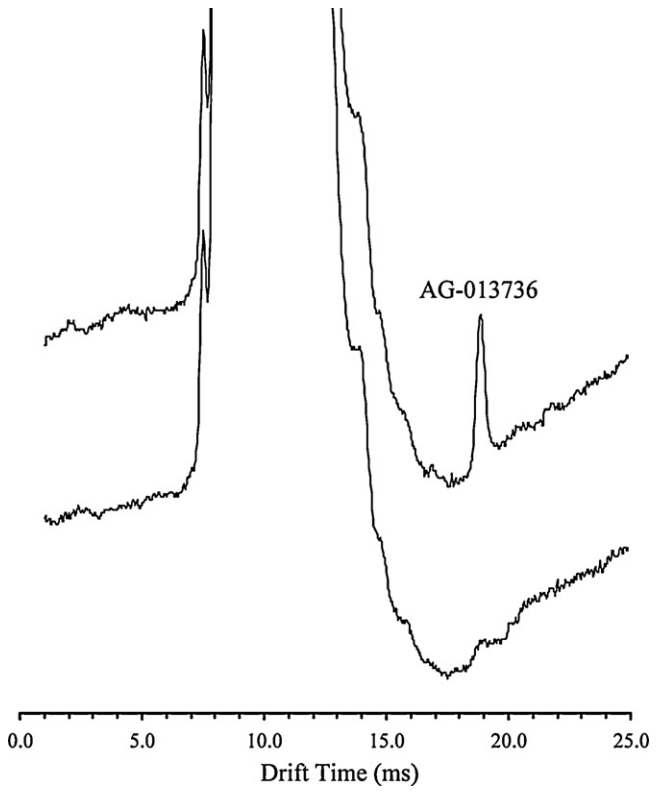

Fig. 3. Representative plasmagrams of a composite sample of 10 placebo tablets spiked with (top) and without (bottom) 10 μg of AG-013736.

3.3. Identification test by DART mass spectrometry

The DART-TOF mass spectrum of AG-013736 was collected in the region of the protonated molecular ion, $[M+H]^+$ where $M = C_{22}H_{18}N_4OS$, and the protonated dimer ion, $[2M+H]^+$. The spectra in both regions were similar to the simulated spectra that were calculated using JEOL's Isotopic Simulator v. 1.3.0.0 program and a resolution of 6000 (FWHM); however, the protonated dimer region was selected for use in the development of an identification test because there were fewer interfering background ion peaks observed in this region. In fact, Table 1 shows that the isotope peaks in the spectrum of the protonated dimer ion of AG-013736 have centroid masses and relative intensities that are within ±3.5 ppm and ±2.6% of the theoretical values, respectively.

As shown in Fig. 4, the mass spectrum of the 1 mg Axitinib tablet matches that of the AG-013736 reference standard. In fact, the four most intense peaks in the active tablet spectrum have masses and relative intensities that are within ±2.6 ppm and ±1.8% of those in the spectrum of the reference standard, respectively. In addition, the spectrum of the placebo tablet shows that there are no interfering excipient-related peaks in this spectral region.

The well-resolved isotope pattern obtainable with a time-of-flight mass spectrometer offers unambiguous identification of AG-013736 in drug substance and tablet samples with multiple degrees of confirmation. However, a high-resolution mass spectrometer is not required for this identification test. For example, the mass spectra of the AG-013736 reference standard and 1 mg Axitinib tablet samples exhibited major peaks with masses and relative intensities that were indistinguishable compared to the resolution of an ion trap mass spectrometer.

Table 1
Isotope peaks in the protonated dimer ion region of the DART-TOF mass spectrum of AG-013736.

Mass (m/z)			Relative intensity (%)	
Measured[a]	Calculated	Error (ppm)[b]	Measured[a]	Calculated
773.2498 (21)	773.2481	2.2	100	100
774.2524 (33)	774.2510	1.8	52.7 (1.2)	52.6
775.2473 (70)	775.2499	−3.5	22.6 (0.5)	23.0
776.2508 (6)	776.2503	0.7	7.4 (0.4)	7.2

[a] Mean and standard deviation based on five measurements.
[b] Error (ppm) = [(measured mass − calculated mass) × 10^6]/(calculated mass).

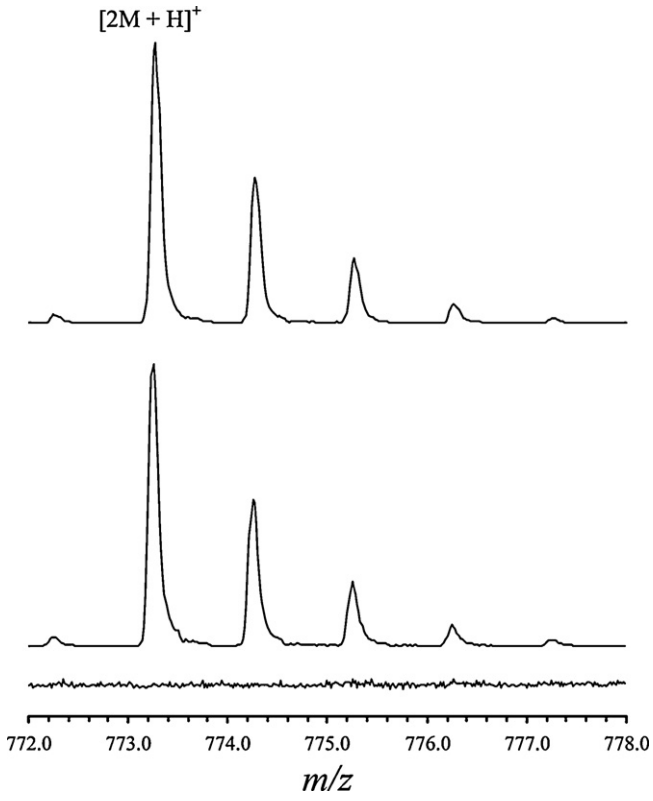

Fig. 4. Representative DART-TOF mass spectra of AG-013736 (top), 1 mg Axitinib tablet (middle), and placebo tablet (bottom) samples. The spectrum of the placebo tablet has been expanded to show that there is no evidence of AG-013736 in this sample.

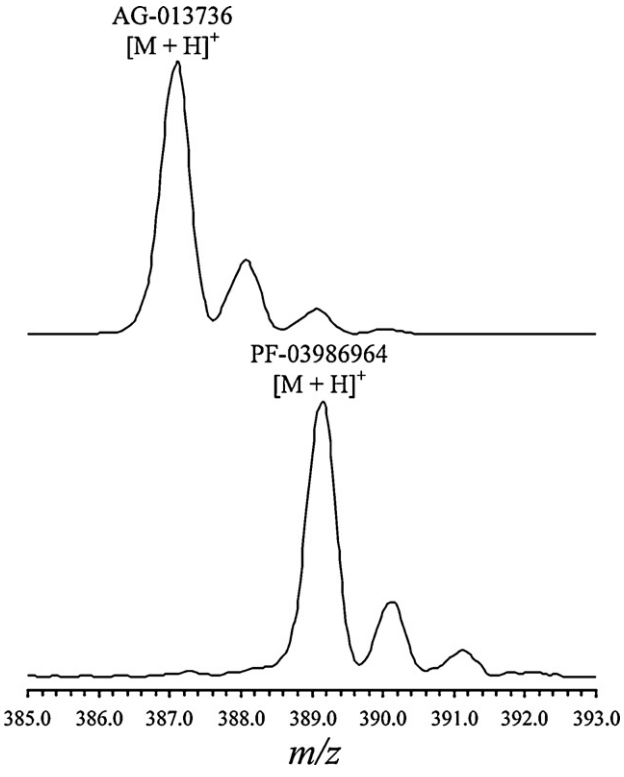

Fig. 5. Representative mass spectra of a 1 mg Axitinib tablet sample (top) and a composite sample of 10 placebo tablets spiked with 10 μg of PF-03986964 (bottom) using DART ion trap mass spectrometry.

3.4. Absence of drug test by DART mass spectrometry

DART-MS can also be used to demonstrate that AG-013736 is not present in the placebo tablets above the ICH reporting threshold of 0.1%. In this case, PF-03986964 was added to the sample solution in order to demonstrate that the melting point capillary was properly positioned between the ionization source and the mass spectrometer and that a compound similar to AG-013736 could be detected at the 0.1% level. In addition, the protonated molecular ion region of AG-013736 and PF-03986964 was used instead of the protonated dimer region in order to achieve the lowest limit of detection possible. As shown in Fig. 5, PF-03986964 was easily detected in this sample; in addition, there was no evidence of the AG-013736 peak at m/z 387 in the bottom spectrum. Therefore, DART-MS can be used as a limit test to demonstrate that AG-013736 is not present in these tablets above this reporting threshold.

4. Conclusions

This study demonstrates that ion mobility spectrometry and DART mass spectrometry can be used as rapid identification methods for AG-013736 in AG-013736 drug substance and 1 mg Axitinib tablet samples with minimal to no sample preparation required prior to analysis. These techniques were also used to develop low-level limit tests in order to demonstrate the absence of drug in matching placebo tablets of Axitinib. Therefore, these methods can be used to verify the presence or absence of AG-013736 in blinded clinical supplies of active and matching placebo tablets of Axitinib. In addition, these methods should be useful for identifying counterfeit Axitinib tablets that do not contain AG-013736.

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References

[1] B.I. Rini, G. Wilding, G. Hudes, W.M. Stadler, S. Kim, J. Tarazi, B. Rosbrook, P.C. Trask, L. Wood, J.P. Dutcher, Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma, J. Clin. Oncol. 27 (2009) 4462–4468.
[2] J.H. Schiller, T. Larson, S.-H.I. Ou, S. Limentani, A. Sandler, E. Vokes, S. Kim, K. Liau, P. Bycott, A.J. Olszanski, J. von Pawel, Efficacy and safety of axitinib in patients with advanced non-small-cell lung cancer: results from a phase II study, J. Clin. Oncol. 27 (2009) 3836–3841.
[3] E.E.W. Cohen, L.S. Rosen, E.E. Vokes, M.S. Kies, A.A. Forastiere, F.P. Worden, M.A. Kane, E. Sherman, S. Kim, P. Bycott, M. Tortorici, D.R. Shalinsky, K.F. Liau, R.B. Cohen, Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study, J. Clin. Oncol. 26 (2008) 4708–4713.
[4] R.J. Kelly, O. Rixe, Axitinib (AG-013736), in: U.M. Martens (Ed.), Small Molecules in Oncology, Recent Results in Cancer Research, vol. 184, Springer-Verlag, Berlin, 2010, pp. 33–44.
[5] R.M. O'Donnell, X. Sun, P.B. Harrington, Pharmaceutical applications of ion mobility spectrometry, Trends Anal. Chem. 27 (2008) 44–53.
[6] R.R. Steiner, R.L. Larson, Validation of the direct analysis in real time source for use in forensic drug screening, J. Forensic Sci. 54 (2009) 617–622.
[7] R.B. Cody, J.A. Laramée, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal. Chem. 77 (2005) 2297–2302.
[8] A.B. Kanu, H.H. Hill Jr., Identity confirmation of drugs and explosives in ion mobility spectrometry using a secondary drift gas, Talanta 73 (2007) 692–699.
[9] M.A. Mäkinen, O.A. Anttalainen, M.E.T. Sillanpää, Ion mobility spectrometry and its applications in detection of chemical warfare agents, Anal. Chem. 82 (2010) 9594–9600.
[10] J.M. Nilles, T.R. Connell, H.D. Durst, Quantitation of chemical warfare agents using the direct analysis in real time (DART) technique, Anal. Chem. 81 (2009) 6744–6749.
[11] F.M. Fernández, C.Y. Hampton, L. Nyadong, A. Navare, M. Kwasnik, Liquid chromatography and ambient ionization time-of-flight mass spectrometry for the analysis of genuine and counterfeit pharmaceuticals, in: I. Ferrer, E.M. Thurman (Eds.), Liquid Chromatography Time-of-Flight Mass Spectrometry: Principles, Tools, and Applications for Accurate Mass Analysis, Wiley, Hoboken, NJ, 2009, pp. 113–132.
[12] J.D. Dunn, C.M. Gryniewicz-Ruzicka, J.F. Kauffman, B.J. Westenberger, L.F. Buhse, Using a portable ion mobility spectrometer to screen dietary supplements for sibutramine, J. Pharm. Biomed. Anal. 54 (2011) 469–474.
[13] C.M. Gryniewicz, J.C. Reepmeyer, J.F. Kauffman, L.F. Buhse, Detection of undeclared erectile dysfunction drugs and analogues in dietary supplements by ion mobility spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 601–606.
[14] D.E. Peterson, G.C. Eden, C.R. Apodaca, D.P. Argyres, A.L. Kennedy, J.G. Barnhill, L.A. Felton, Ion mobility spectrometry for determination of active drug in blinded dosage forms, AAPS Newsmagazine (2005) 18–19, http://www.aapspharmaceutica.com/publications/newsmagazine/2005/feb05/default.asp, (accessed 11.02.11).
[15] H. Borsdorf, G.A. Eiceman, Ion mobility spectrometry: principles and applications, Appl. Spectrosc. Rev. 41 (2006) 323–375.
[16] G.A. Eiceman, Z. Karpas, Ion Mobility Spectrometry, second ed., CRC Press, Boca Raton, FL, 2005.
[17] J.A. Laramee, R.B. Cody, Chemi-ionization and direct analysis in real time (DART™) mass spectrometry, in: M.L. Gross, R.M. Caprioli (Eds.), The Encyclopedia of Mass Spectrometry, vol. 6, Elsevier, Amsterdam, 2007, pp. 377–387.
[18] S. Bender, D.J. Skalitzky, D. Hu-Lowe, Indazole compounds and pharmaceutical compositions for inhibiting protein kinases, and methods for their use, U.S. Pat. Appl. Pub., U.S. 2005/0038097 A1, February 17, 2005.
[19] L. Song, S.C. Gibson, D. Bhandari, K.D. Cook, J.E. Bartmess, Ionization mechanism of positive-ion direct analysis in real time: a transient microenvironment concept, Anal. Chem. 81 (2009) 10080–10088.
[20] R.A. Singer, manuscript in preparation.
[21] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q3B(R2), Impurities in New Drug Products, 2006, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html, (accessed 11.02.11).