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Detection of undeclared erectile dysfunction drugs and analogues in dietary supplements by ion mobility spectrometry

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ABSTRACT

An ion mobility spectrometry (IMS) method was developed to screen for the presence of undeclared synthetic erectile dysfunction (ED) drugs or drug analogues in herbal dietary supplements claiming to enhance male sexual performance. Ion mobility spectra of authenticated reference materials including three FDA approved drugs (sildenafil citrate, tadalafil, vardenafil hydrochloride trihydrate) and five previously identified synthetic analogues (methisosildenafil, homosildenafil, piperidenafil, thiosildenafil, thiomethisosildenafil) were measured to determine their reduced ion mobilities (K_0). All eight compounds exhibited reduced mobilities between 0.8257 and 1.2876 $cm^2/(V\,s)$. Twenty-six herbal products were then screened for the presence of these compounds, and 15 of the 26 products tested positive for the presence of ED drug or drug analogue adulterants based on their reduced ion mobilities. IMS results were compared against the results obtained from an independent LC/MS reference method for the identical samples. Herbal dietary supplements containing adulterants were classified with 100% accuracy and most of the adulterants were correctly identified by a comparison of the K_0 of the adulterant to the K_0 of the authenticated reference material. The results demonstrate that IMS is a viable method for screening herbal dietary supplements for the presence of ED drug or drug analogue adulterants.

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1. Introduction

Dietary supplements and herbal remedies are increasing in popularity all over the world due to the widespread belief that natural products are safer and healthier than synthetic ingredients. Natural supplements are widely available and claim to benefit consumers by treating various health conditions as well as promoting general well being. One of the major concerns related to the safety of dietary supplements is their adulteration with undeclared synthetic pharmaceutical products in order to enhance the claims stated on the label.

In recent years, there have been several reports on the detection of synthetic erectile dysfunction (ED) drugs in herbal dietary supplements claiming to enhance male sexual performance. Herbal products have been adulterated with not only the three FDA approved ED drugs (sildenafil citrate, tadalafil, vardenafil hydrochloride trihydrate) [1–5], but also with synthetic analogues of these drugs, such as piperidenafil [6], acetildenafil [3,7–10], methisosildenafil [11], homosildenafil [1,3,8,10,12], hydroxyhomosildenafil [3,8,10,12], thiohomosildenafil [13], thiosildenafil [13,14], and thiomethisosildenafil [14], in which minor modifications were made to the molecular structure of one of the approved ED drugs. The presence of these analogues as illicit adulterants in herbal products poses a serious health risk to consumers as their efficacy and safety have not been clinically proven and may result in unpredictable adverse effects. Undeclared ED drugs in these natural products can also interact with other prescription medications and can result in adverse effects, some of which could be life threatening. Patients with pre-existing medical conditions, including those with heart problems, those taking heart medications, or those at risk for strokes, may be at an increased risk of serious health effects [3,15].

LC/MS is the most common method currently utilized for the screening of adulterants in herbal dietary supplements [1–14]. However, due to its instrument set up time and extended measurement times it is not well suited for the rapid screening of adulterants in herbal products. Ion mobility spectrometry (IMS) is a high throughput separation method that characterizes chemical substances based upon their gas phase ion mobilities. The drift velocity, v_d, of a gas phase ion swarm that drifts under the influence of an electric field, E, against a counterflow of a neutral buffer gas is

$$v_d = K \cdot E \quad (1)$$

where K is the ion mobility. The drift velocity is inversely proportional to the buffer gas density, and therefore K depends on the buffer gas pressure and temperature. The reduced ion mobility is

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defined as

$$K_0 = \frac{L}{t_d E}\frac{P}{760}\frac{273.2}{T} \quad (2)$$

where L is the length of the drift tube, t_d is the time required for the ion to drift to the detector, P is the buffer gas pressure in Torr and T is the buffer gas temperature in Kelvin. When P = 760 Torr and T = 273.2 K, $K_0 = L/t_d E$. Ion mobilities also depend on the size, shape, and charge of a molecular ion, and can often be used for qualitative identification of an analyte. From Eq. (2) it is apparent that $K_0 \cdot t_d$ is constant during the measurement of an ion mobility spectrum in a well-controlled instrument. Therefore an internal calibrant with known ion mobility, $K_{0,C}$, can be used to provide accurate measurement of an analyte's ion mobility, $K_{0,A}$, from its drift time, $t_{d,A}$, and the drift time of the internal calibrant, $t_{d,C}$ using the equation

$$K_{0,A} = K_{0,C} \cdot \frac{t_{d,C}}{t_{d,A}}. \quad (3)$$

The use of the internal calibrant obviates the influence of small variations in P, T and E that can occur between measurements made on different days, and allows reduced ion mobilities to be used for qualitative analysis of analytes when analyte reference materials are available.

IMS instruments are portable, easy to use, and unlike HPLC do not require solvents or column materials. In forensic sciences, IMS has been used as a detection device for the presence of trace amounts of illicit drugs [16–21] or explosives [16,22–25]. Ion mobility methods currently employed in the pharmaceutical industry have been used for quality assurance and process monitoring [17]. IMS instruments have been used for cleaning verification of manufacturing equipment [17,26], direct formulation analysis of pharmaceutical products for use in clinical trials or authentication purposes [17,27,28], and environmental monitoring of the air quality in manufacturing facilities [16,17,29].

IMS is an ideal screening tool for the detection of adulterants in ED enhancement dietary supplements due to its high speed, selectivity, and low detection limits. ED drugs and their analogues have complex molecular structures that contain amine groups as illustrated in Fig. 1. Amines generally provide an intense and interference free response in IMS due to their high proton affinities. Amines protonate readily in the IMS source and the lone pair of the nitrogen electron is the favored site for protonation. Since the major ionic product in most cases is the protonated molecular ion, its detection can be used to easily identify the presence of an ED drug or its analogue. The purpose of this paper is to demonstrate the feasibility of IMS as a rapid screening method for synthetic erectile dysfunction drugs in herbal dietary supplements.

2. Experimental

2.1. Materials

FDA approved ED drug reference standards were acquired from different manufacturers. Sildenafil citrate was obtained from Pfizer (New York, NY, USA), tadalafil from Lilly (Indianapolis, IN, USA), and vardenafil hydrochloride trihydrate from Bayer (Wayne, NJ, USA). Authenticated reference samples of homosildenafil, methisosildenafil, piperidenafil, thiomethisosildenafil and thiosildenafil were extracted from herbal dietary supplements, and identified using LC/MS, CID-MS^n, NMR, and hydrolytic techniques. The mass spectral and NMR analyses of these compounds have been described in detail elsewhere [6,7,11,14]. HPLC-grade isopropyl alcohol (IPA) and methanol (MeOH) were purchased from EM Science (Gibbstown, NJ, USA).

Table 1
IMS operating conditions.

Ion mode	Parameter	Setting
Positive	Drift tube temperature (°C)	232
	Inlet temperature (°C)	289
	Desorber temperature (°C)	291
	Calibrant temperature (°C)	78
	Drift flow (cc/min)	300
	Analysis time (s)	12
	Pulse width (ms)	0.2
	Scan period (ms)	25
	Scan number per segment	10
	Segment number per analysis	48

2.2. Reference and sample preparation

Reference samples, that include approved ED drug reference standards and authenticated ED drug analogue reference materials, were prepared in IPA with concentrations of 0.30–0.45 μg/ml. Composites of herbal dietary supplements were prepared by mixing the contents of 2–3 capsules and then vortexing briefly to provide a uniform mixture. Approximately 2–3 mg of each composite was extracted with 10 ml MeOH in a glass tube sealed with a PTFE-lined cap. The mixture was vortexed vigorously for 30 s, placed in an ultrasonic bath for 10 min, vortexed and sonicated a second time, vortexed a third time and centrifuged at room temperature for 15 min at 3300 rpm (Centrifig, model 228, bench-top centrifuge Fisher Scientific, Pittsburgh, PA, USA). The supernatant liquid was decanted to a second test tube and dilutions were prepared at 1:10, 1:50, and 1:250 with IPA.

2.3. Ion mobility spectrometry

An IONSCAN®-LS (Smiths Detection, Danbury, CT, USA) ion mobility spectrometer operated in the positive mode was used to screen the samples for the presence of synthetic ED drugs and their analogues. The operating conditions are detailed in Table 1. The IONSCAN®-LS system was equipped with an internal nicotinamide calibrant that was injected into the spectrometer with every IMS measurement. Ion mobility spectra were recorded using the IONSCAN®-LS IM-Station Software version: 5.389. A 1 μl sample was deposited onto a Teflon substrate using an autosampler and the volatile solvents were allowed to evaporate. The substrate was then introduced into the IMS system and placed on the desorber heater. Analyte molecules were vaporized and carried from the heated inlet to the ionization chamber in a flow of dry air carrier gas. The volatilized analyte molecules were selectively ionized by a ^{63}Ni β source in a controlled chemical ionization environment to produce molecular ions. The positive analyte ions were gated into the drift tube every 25 ms with a pulse width of 0.2 ms, where they were accelerated under an applied electric field toward a collector electrode against a counterflow of approximately 1 atm of dry air. Analyte ion drift times were measured relative to the drift time of the instrument's internal nicotinamide calibrant. The nicotinamide drift time was found to have a standard deviation of 2–4 μs over the course measurements made on multiple runs on a single day, for a relative standard deviation (R.S.D.) of 0.03–0.04%. Replicate analyte drift times measured on the same day exhibited similar R.S.D.s. The nicotinamide calibrant drift time measured on 4 different days separated by weeks or months had an R.S.D. of 0.6%, however the ratio of the calibrant to analyte drift times for each run over these same measurements had R.S.D.s between 0.02% and 0.07%, indicating that Eq. (3) holds for this instrument.

Analyte bands were identified visually and user-selected for analysis by the IM-Station Software. The software fit the selected bands to Gaussian band shapes and reported the band peak drift

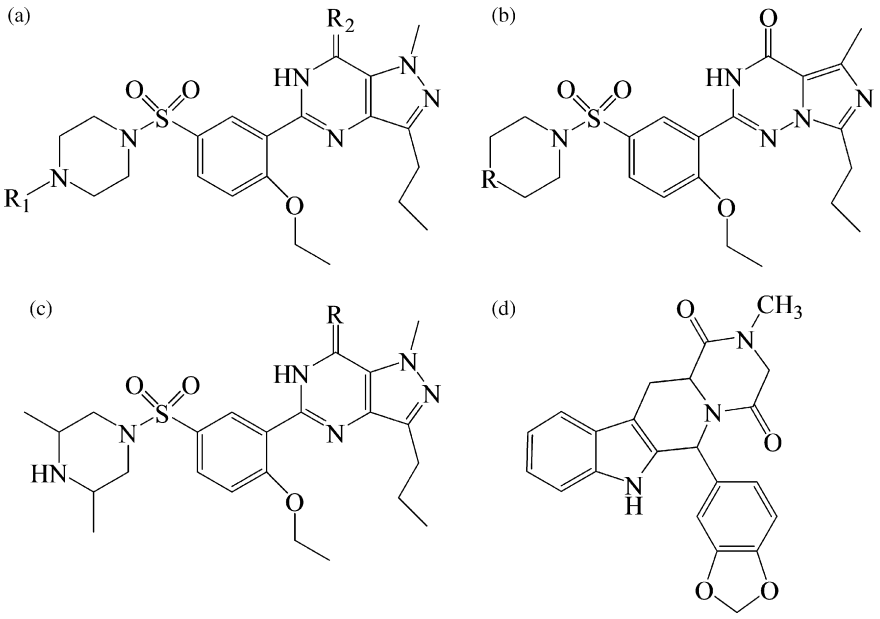

Fig. 1. Chemical structures of ED drugs and their analogues: (a) sildenafil [$R_1 = CH_3$ $R_2 = O$], homosildenafil [$R_1 = CH_3CH_2$ $R_2 = O$], thiosildenafil [$R_1 = CH_3$ $R_2 = S$], (b) vardenafil [$R = CH_3CH_2N$], piperidenafil [$R = CH_2$], (c) methisosildenafil [R = O], thiomethisosildenafil [R = S] and (d) tadalafil.

Table 2
IMS characteristics of ED drug standards and reference analogue samples.

Reference	K_0 ($cm^2/(V\,s)$)	LOD (ppm)	$[MH]^+$ m/z
Thiomethisosildenafil	0.8559 ± 0.0003	0.2	505
Thiosildenafil	0.8761 ± 0.0005	0.2	491
Homosildenafil	0.8617 ± 0.0003	0.2	489
Methisosildenafil	0.8617 ± 0.0002	0.15	489
Vardenafil	0.8254 ± 0.0004	0.2	489
Sildenafil	0.8774 ± 0.0004	0.15	475
Piperidenafil	0.8695 ± 0.0003	0.15	460
Tadalafil[a]	1.0121 ± 0.0006	0.4	390
Tadalafil[a]	1.2876 ± 0.0009		268

[a] Tadalafil displays two stable ions.

time (t_d), full width at half maximum, amplitude, and area, and computed the reduced ion mobility ($K_{0,A}$) from the band peak drift time. Identification of an analyte was based on its characteristic $K_{0,A}$ ($cm^2/(V\,s)$). $K_{0,A}$ was measured relative to the known reduced ion mobility of the internal nicotinamide calibrant, $K_{0,C}$, using Eq. (3). $K_{0,C}$ for nicotinamide was set to a value of 1.8614 $cm^2/(V\,s)$ for this study, and was assumed to be exact. From Eq. (3) it is apparent that the error in $K_{0,A}$ is propagated from the error in the measured quantity $t_{d,C}/t_{d,A}$, the ratio of the calibrant drift time to the analyte drift time. This ratio was measured four times over the course of several months for each ED drug reference material examined in this study, and the relative standard deviations of these measurements ranged from 0.02% to 0.07%. The average ion mobilities and standard deviations from these measurements are reported in Table 2. These results indicate that analyte ion mobilities can be determined accurately to 4 decimal places.

3. Results

Eight reference materials dissolved in IPA were evaluated using IMS. The chemical structures of these compounds are displayed in Fig. 1. The ion mobility spectra for an IPA blank and three ED drug reference materials are shown in Fig. 2. The sharp peak observed at approximately 9.5 ms in all ion mobility spectra was attributed to the instrument's internal nicotinamide calibrant. All ion mobilities were calibrated to the drift time of this peak as described above. An impurity peak attributed to the IPA background was observed at approximately 8.3 ms with a reduced mobility of 2.1051 $cm^2/(V\,s)$. Protonated ion peaks for ED drug reference materials evaluated in this work were observed at drift times ranging from 13.5 to 21.5 ms, corresponding to reduced mobilities ranging from 1.2876 to 0.8257 $cm^2/(V\,s)$. Two stable product ion peaks at 13.5 and 17.1 ms were observed in the tadalafil ion mobility spectrum; whereas the remaining seven ED drug reference materials resulted in one product ion peak that could be assigned to the protonated molecular ions as illustrated in the ion mobility spectra of sildenafil and piperidenafil in Fig. 2. The observation of two peaks in the tadalafil spectrum indicated that tadalafil underwent fragmentation during the chemical ionization process. The product ion peak with the shorter drift time ($K_0 = 1.2876\ cm^2/(V\,s)$) could be attributed to a stable fragment of the tadalafil molecule (m/z 268) that has underwent the loss of a benzodioxole moiety [3]; whereas the peak observed at a longer drift time ($K_0 = 1.0121\ cm^2/(V\,s)$) was attributed to the parent ion (m/z 390). Similar fragmentation behavior for tadalafil has been observed in LC/MS analysis [3]. ED drug reference materials were analyzed in triplicate on four different days over several months,

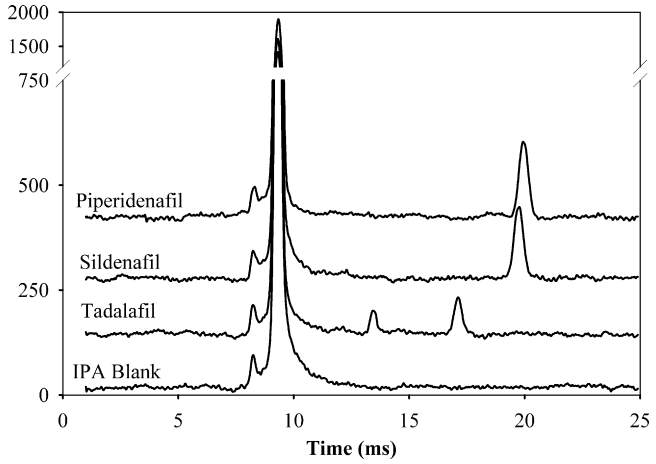

Fig. 2. Ion mobility spectra of tadalafil, sildenafil and piperidenafil in isopropyl alcohol. An isopropyl alcohol blank is also shown. The spectra have been offset for clarity.

and the average ion mobilities and their standard deviations are reported in Table 2. Sample dilutions for each reference material were prepared at 0.05 ppm increments for sample concentrations between 0.10 and 0.45 ppm in order to determine the IMS detection limits for ED drugs and their analogues. LODs for the ED drug reference materials were determined as the concentration that resulted in a signal-to-noise ratio of 3.

Composite samples of 26 herbal dietary supplements were extracted using the simple methanol extraction method described above. The extraction procedure reduced the contribution of interferants associated with the complex herbal matrices to the ion mobility spectrum. To account for potential variability of adulterant concentrations, the supernatant liquids from the extracts were diluted by 10×, 50× and 250×. The more dilute sample preparations were analyzed first in order to prevent saturation of the IMS ionization chamber with high analyte concentrations. Ion peaks detected in the more dilute sample preparations were verified in the analysis of the next less dilute extract. Herbal products having a positive adulterant confirmation at the 250× dilution were not analyzed at the 10× dilution in order to prevent sample overload of the ionization chamber. Herbal dietary supplements analyzed using IMS were classified as adulterated products when a peak or multiple peaks having reduced ion mobilities in the range of the ion mobilities of the ED drug reference materials were observed. Non-adulterated products were identified by the absence of peaks with ion mobilities in the range of the ED drug reference materials. The accuracy of the classification of herbal dietary supplements as either adulterated or not adulterated was 100%. In other words, all supplements that were suspected of adulteration based on their ion mobility spectra were found to be adulterated by the more labor intensive LC/MS methods, and all supplements that were classified as unadulterated by IMS were found to be unadulterated by LC/MS.

The ion mobility spectra for several extracted herbal dietary supplements are shown in Fig. 3. Samples S-3 and S-14 were found to contain no ED drug adulterants by LC/MS, and their ion mobility spectra also exhibited no peaks at drift times associated with the ED drugs listed in Table 2. The spectrum of sample S-3 showed two peaks at drift times of 10.7 and 15.7 ms, and these peaks could be attributed to unknown compounds extracted from the herbal supplement matrix. Sample S-15 had a strong peak at about 20.1 ms, corresponding to an ion mobility of 0.8694 $cm^2/(V\,s)$. The closest ion mobility was displayed by piperidenafil (Table 2), and this assignment was consistent with the reference LC/MS analysis of S-15. The ion mobility spectrum of sample S-26 showed peaks at approximately 19.9, 17.2 and 13.6 ms that could be attributed to sildenafil and tadalafil by comparison to the spectra in Fig. 2. The S-26 spectrum also had two peaks at 11.8 and 12.9 ms that could not be attributed to any of the reference materials listed in Table 2, and were probably attributable to extractable compounds in the herbal dietary supplement matrix.

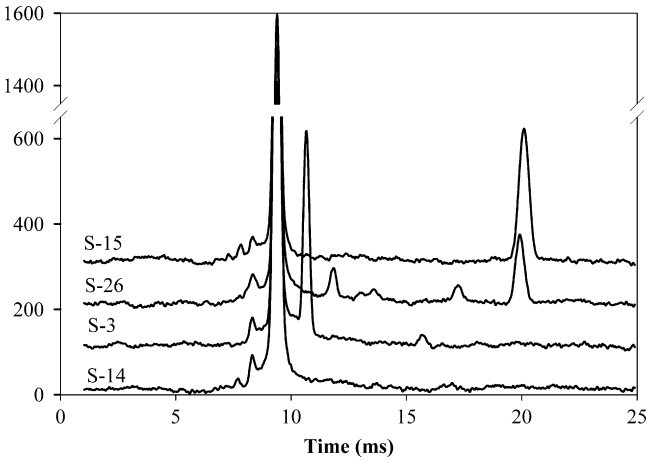

Fig. 3. Ion mobility spectra of herbal dietary supplement extracts diluted in isopropyl alcohol. The compositions of the samples are given in Table 3. Spectra are offset for clarity.

The ion mobility spectra of fifteen of the tested herbal products exhibited one or more molecular ion peaks with ion mobilities in the range associated with the ED drug reference materials. Table 3 lists the calculated reduced ion mobilities of the analytes observed in the 26 herbal dietary supplements examined in this study. Qualitative identification of the adulterants in the dietary supplements was made by comparing the reduced mobilities of the analytes in Table 3 to the reduced mobilities of the ED drug reference materials in Table 2. Qualitative identification by IMS was confirmed by independent LC/MS experiments [6,7,11,14] for the identical herbal dietary supplements. Adulterants were correctly identified by IMS, with the exception of samples containing either homosildenafil or methisosildenafil. Though the IMS measurements correctly identified the adulterant as being one of these two substances, these compounds were indistinguishable by IMS due to the similarity of their ion mobilities. Supplements testing positive for undeclared adulterants generally contained a single adulterant with the exception of herbal product S-26, which contained both sildenafil and tadalafil.

4. Discussion

IMS analysis of herbal dietary supplements extracted in methanol resulted in simple spectral profiles in which ED drug adulterants exhibited intense protonated molecule peaks. Protonated molecule peaks observed with drift times of 19–21 ms in the ion mobility spectra could be used as markers for the presence of known ED drug adulterants whose structures are similar to sildenafil and vardenafil. The molecular structure of tadalafil differs greatly from the other ED drug adulterants studied in this work. Tadalafil is the lightest ED drug molecule and its ion mobility spectrum resulted in two stable ion peaks with shorter drift times and greater reduced mobilities when compared to the drift times and mobilities of the other ED drug adulterants. Ions associated with extractable compounds from the herbal matrices of several dietary supplements were also observed at drift times in the region of the tadalafil ions. These samples were correctly classified as not adulterated because of slight differences in the reduced mobilities when compared to the mobilities of the tadalafil ions, but also because only one peak was observed within this drift region of the mobility spectra. Thus, structural information can be derived from the presence of the fragment ion, but more importantly, the fragment ion together with the molecular ion can serve as markers for the tadalafil adulterant.

The data presented in this work demonstrated the capability of IMS as a rapid screening tool for the detection of ED drug adulterants in herbal dietary supplements. Furthermore, in most cases the calculated adulterant ion mobilities were used to qualitatively identify the adulterant. In some cases, parent ions of the same mass could be separated, because ions of the same mass but different functional groups or ions of the same functional groups but different geometrical arrangements could exhibit different reduced mobilities, reflecting the influence of shape and size on mobility. For example, homosildenafil and vardenafil are constitutional isomers that demonstrate how structural variations can influence an ion's mobility. The two analytes have similar chemical structures with the exception of a nitrogen position in the 5-membered ring, as illustrated in Fig. 1. The reduced mobilities for homosildenafil and vardenafil were 0.8617 and 0.8254 $cm^2/(V\,s)$, respectively. The two isomers differed in drift time by 0.9 ms and the mobility of

Table 3
Measured ion mobilities of extracts from 26 herbal products, and identification of adulterants based on the reference ion mobilities.

Sample	Mobility ($cm^2/(V s)$)			ED Drug
	250×	50×	10×	
S1	0.8617 ± 0.0004	0.8620 ± 0.0002		Methisosildenafil[a]/homosildenafil
S2	0.8628 ± 0.0013	0.8619 ± 0.0001		Methisosildenafil[a]/homosildenafil
S3			1.6383 ± 0.0002	None
S4			1.6376 ± 0.0010	None
S5		1.0334 ± 0.0004	1.0320 ± 0.0002	None
S6				None
S7		1.0325 ± 0.0009	1.0326 ± 0.0005	None
S8		0.8774 ± 0.0005		Sildenafil
S9				None
S10		1.0325 ± 0.0014	1.0326 ± 0.0002	None
S11		0.8614 ± 0.0001		Homosildenafil[a]/methisosildenafil
S12	0.8624 ± 0.0008	0.8618 ± 0.0003		Methisosildenafil[a]/homosildenafil
S13		0.8696 ± 0.0001		Piperidenafil
S14				None
S15			0.8694 ± 0.0002	Piperidenafil
S16	0.8614 ± 0.0011	0.8627 ± 0.0005		Methisosildenafil[a]/homosildenafil
S17		0.8766 ± 0.0005	0.8762 ± 0.0001	Thiosildenafil
S18	0.8621 ± 0.0002	0.8618 ± 0.0001		Methisosildenafil[a]/homosildenafil
S19		0.8560 ± 0.0002		Thiomethisosildenafil
S20		0.8565 ± 0.0006		Thiomethisosildenafil
S21				None
S22				None
S23	0.8779 ± 0.0003	0.8774 ± 0.0001		Sildenafil
S24				None
S25[b]		1.2762 ± 0.0005	0.8557 ± 0.0005	Thiomethisosildenafil
			1.2761 ± 0.0002	
S26[c]			0.8771 ± 0.0003	Sildenafil
			1.0130 ± 0.0001	Tadalafil
			1.2865 ± 0.0021	

[a] Indicates which of these two ED drugs or analogues was identified by LC/MS.
[b] Two compounds were detected in this sample by IMS. One was thiomethisosildenafil and the other was outside the ion mobility range of an ED drug or analogue.
[c] Both sildenafil and tadalafil were identified in this sample.

vardenafil suggests that the molecule has a larger cross-sectional area that may be influenced by either the nitrogen positions or the arrangement of the double bonds within the ring structures. On the other hand, structural changes do not always influence the mass, shape, or size of a molecule. For example, homosildenafil and methisosildenafil have the same mass to charge ratio (m/z) but vary in the distribution of methyl groups on their piperazine rings. Methisosildenafil has two methyl groups attached to the carbon atoms while homosildenafil has an ethyl group attached to the nitrogen. In this case, the two analogues had similar reduced mobilities, indicating that the configuration of the methyl groups did not significantly alter the molecular shape. Thus the appearance of a peak in the ion mobility spectrum with a reduced mobility of 0.8617 $cm^2/(V s)$ indicated the presence of one of these two ED drug adulterants, but other analytical methods would be required to unambiguously identify its structure.

Ion mobility measurements cannot be described in terms of mass resolution due to the effects of size and shape on mobility. In IMS, separation efficiencies are typically reported as resolving power (R_p) and are measured as the ratio of the ion's drift time (t_d) to its peak width at half height, which measures the sharpness of the ion pulse. Typical peak full width at half maxima were 0.475 ms for the substances listed in Table 2, and resolving power varied from 28.5 for the sildenafil ion to 44.5 for vardenafil using the operating parameters listed in Table 1. R_p is related to the number of theoretical plates, N, and for the IMS method can be determined by the following equation [30]:

$$N = 5.55(R_p)^2 \quad (4)$$

The value of N achieved for the adulterants examined in this study ranged from 4500 for the sildenafil ion to 10,100 for vardenafil. The method resolution can be determined from Eq. (5),

$$R = \frac{R_p}{1.70}\frac{\alpha - 1}{\alpha} = \frac{\sqrt{N}}{4}\frac{\alpha - 1}{\alpha} \quad (5)$$

where R is the resolution and α is the selectivity factor defined as $K_{01}/K_{02} = t_{d,2}/t_{d,1}$ [30]. When the signal-to-noise ratio of the spectrum exceeds 10, a resolution of 0.6 is adequate to resolve two peaks and measure accurate drift times. Typical ion mobility spectra measured in this work such as those shown in Figs. 2 and 3 exhibited signal-to-noise ratios that exceed 20, and therefore we anticipate that any pair of ED drug substances listed in Table 2 with a resolution greater than 0.6 could be resolved. Of the 36 distinct pairs of ions represented in Table 2 (including the tadalafil fragment), 21 of them would be resolved under these assumptions, and 15 of them would not be resolved. Although IMS can accurately screen for the presence of ED drug adulterants in herbal dietary supplements, it is not capable of independent determination of molecular structure without the use of reference materials or standards for ion mobility comparison. In this respect it is similar to qualitative analysis using HPLC. However, the minimal sample preparation and analysis time for IMS allows higher throughput and rapid decision analysis compared with HPLC. Furthermore, portable IMS instruments are currently available that can support the development of field deployable methods for rapid screening. Methods for introduction of solid samples into the IMS spectrometer are under investigation, and such methods would further reduce sample preparation time.

5. Conclusion

Ion mobility spectrometry has been shown to be a rapid, selective, sensitive, and reliable screening method for the detection and identification of illicit ED drugs adulterants in herbal dietary supplements. The method was successfully applied to the analysis of

26 commercially available herbal dietary supplements. Among the 26 samples tested, 15 were found to contain adulterants. Herbal supplements containing adulterants were classified with 100% accuracy by a comparison of the reduced mobilities of the ED drug adulterant in the herbal product to the ED drug reference materials. While no adulterant was misidentified, two of those analyzed could not be differentiated by IMS. The adulteration of dietary supplements by ED drug analogues appears to be a growing trend. Consequently, screening for adulterants should not be restricted to approved ED drugs or previously identified analogues since new analogues are always being developed.

References

[1] S.R. Gratz, C.L. Flurer, K.A. Wolnik, J. Pharm. Biomed. Anal. 36 (2004) 525–533.
[2] Q. Liang, J. Qu, G. Luo, Y. Wang, J. Pharm. Biomed. Anal. 40 (2006) 305–311.
[3] P. Zou, S.S.-Y. Oh, P. Hou, M.-Y. Low, H.-L. Koh, J. Chromatogr. A 1104 (2006) 113–122.
[4] N. Fleshner, M. Harvey, H. Adomat, C. Wood, A. Eberding, K. Heresy, E. Guns, J. Urol. 174 (2005) 636–641.
[5] X. Zhu, S. Xiao, B. Chen, F. Zhang, S. Yao, Z. Wan, D. Yang, H. Han, J. Chromatogr. A 1066 (2005) 89–95.
[6] J.C. Reepmeyer, J.T. Woodruff, J. Chromatogr. A 1125 (2006) 67–75.
[7] J.C. Reepmeyer, J.T. Woodruff, J. Pharm. Biomed. Anal. 44 (2007) 887–893.
[8] S.S-Y. Oh, P. Zou, M.-Y. Low, H.-L. Koh, J. Toxicol. Environ. Health Part A 69 (2006) 1951–1958.
[9] B.J. Venhuis, L. Blok-tip, D. de Kaste, Forensic Sci. Int. 177 (2008) e25–e27.
[10] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Hamzink, J. ten Hove, M. Vredenbregt, D. de Kaste, Food Addit. Contam. 21 (2004) 737–748.
[11] J.C. Reepmeyer, J.T. Woodruff, D.A. d'Avignon, J. Pharm. Biomed. Anal. 43 (2007) 1615–1621.
[12] H.J. Park, H.K. Jeong, M.I. Chang, M.H. IM, J.Y. Jeong, D.M. Choi, K. Park, M.K. Hong, J. Youm, S.B. Han, D.J. Kim, J.H. Park, S.W. Kwon, Food Addit. Contam. 24 (2007) 122–129.
[13] P. Zou, P. Hou, S.-Y. Oh, Y.M. Chong, B.C. Bloodworth, M.-Y. Low, H.-L. Koh, J. Pharm. Biomed. Anal. 47 (2008) 279–284.
[14] J.C. Reepmeyer, D.A. d'Avignon, J. Pharm. Biomed. Anal 49 (2009) 145–150.
[15] E. Wespes, E. Amar, D. Hatzichristou, F. Montorsi, J. Pryor, Y. Vardi, Eur. Urol. 41 (2002) 1–5.
[16] G.A. Eiceman, Z. Karpas, Ion Mobility Spectrometry, 2nd ed., CRC Press, Boca Raton, FL, 2005.
[17] R.M. O'Donnell, X. Sun, P.B. Harrington, Trends Anal. Chem. (2008).
[18] T. Keller, A. Keller, E. Tutsch-Bauer, F. Monticelli, Forensic Sci. Int. 161 (2006) 130–140.
[19] A. Miki, T. Keller, P. Regenscheit, R. Dirnhofer, M. Tatsuno, M. Tatagi, M. Nishikawa, H. Tsuchihashi, J. Chromatogr. B 692 (1997) 319–328.
[20] T. Keller, A. Miki, P. Regenscheit, R. Dirnhofer, A. Schneider, H. Tsuchihashi, Forensic Sci. Int. 94 (1998) 55–63.
[21] A.H. Lawrence, Anal. Chem. 58 (1986) 1269–1272.
[22] P. Kolla, Anal. Chem. 67 (1995) 184A–189A.
[23] F. Garofolo, F. Marziali, V. Migliozzi, A. Stama, Rapid Commun. Mass Spectrom. 10 (1996) 1321–1326.
[24] J.I. Baumbach, Anal. Bioanal. Chem. 384 (2006) 1059–1070.
[25] J.M. Perr, K.G. Furton, J.R. Almirall, J. Sep. Sci. 28 (2005) 177–183.
[26] R. DeBono, S. Stefanou, M. Davis, G. Walia, Pharm. Technol. 26 (2002) 72–78.
[27] D.E. Peterson, G.C. Eden, C.R. Apodaca, D.P. Argyres, A.L. Kennedy, AAPS Newsmag. (2005) 18–19.
[28] G.A. Eiceman, D.A. Blyth, D.B. Shoff, P.A. Snyder, Anal. Chem. 62 (1990) 1374–1379.
[29] G.A. Eiceman, S. Sowa, S. Lin, S.E. Bell, J. Hazard. Mater. 43 (1995) 13–30.
[30] A.B. Kanu, P.E. Haigh, H.H. Hill Jr., Anal. Chim. Acta 553 (2005) 148–159.