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Using a portable ion mobility spectrometer to screen dietary supplements for sibutramine☆

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ABSTRACT

In response to recent incidents of undeclared sibutramine, an appetite suppressant found in dietary supplements, we developed a method to detect sibutramine using hand-held ion mobility spectrometers with an analysis time of 15 s. Ion mobility spectrometry is a high-throughput and sensitive technique that has been used for illicit drug, explosive, volatile organic compound and chemical warfare detection. We evaluated a hand-held ion mobility spectrometer as a tool for the analysis of supplement extracts containing sibutramine. The overall instrumental limit of detection of five portable ion mobility spectrometers was 2 ng of sibutramine HCl. When sample extractions containing 30 ng/μl or greater of sibutramine were analyzed, saturation of the ionization chamber of the spectrometer occurred and the instrument required more than three cleaning cycles to remove the drug. Hence, supplement samples suspected of containing sibutramine should be prepared at concentrations of 2–20 ng/μl. To obtain this target concentration range for products containing unknown amounts of sibutramine, we provided a simple sample preparation procedure, allowing the U.S. Food and Drug Administration or other agencies to screen products using the portable ion mobility spectrometer.

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1. Introduction

Unlike prescription and over-the-counter medications, dietary supplements do not require U.S. Food and Drug Administration (FDA) approval. Thus, supplement products are susceptible to adulteration, contamination or mislabelling. Between 2008 and 2009, undeclared drugs, including bumetanide, cetilistat, fenproporex, fluoxetine, furosemide, phenytoin, rimonabant and sibutramine, were found in over 70 weight loss supplements [1]. Adverse health events associated with those consuming these undeclared drugs can occur, especially when the dose is above that of prescription form.

Sibutramine hydrochloride monohydrate, brand name MERIDIA®, is used to treat obesity and is legally available in the U.S. by prescription. Each MERIDIA® capsule contains 5 mg, 10 mg or 15 mg of sibutramine hydrochloride monohydrate. Recently, sibutramine was found in counterfeit Alli, an over-the-counter product marketed for weight loss whose active ingredient is orlistat. Sibutramine has been detected in unapproved weight loss products at levels of 0.1–40 mg per capsule,[1] the latter amount being about three times more than the U.S. FDA approved dose. In the laboratory, evaluation of such products is typically performed by gas chromatography-mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) with detection by UV, mass spectrometry (MS) or tandem MS (MS/MS). Using these techniques, analysis times are on the order of 15–75 min [2–11], which can be costly when multiple samples are tested. Additionally, GC requires conversion of sibutramine hydrochloride to the free base form prior to analysis [2–4], which results in increased sample preparation time and possible loss of analyte. Ion mobility spectrometry (IMS) is a rapid, sensitive technique that requires minimal sample preparation, thereby making it a viable screening tool for testing pharmaceuticals [12–20]. Hand-held IMS instruments have been used mainly for illicit drug, explosive, volatile

Abbreviations: CL, critical level; DPA, Division of Pharmaceutical Analysis; DU, digital unit; ED, erectile dysfunction; ESI-MS, electrospray ionization mass spectrometry; FDA, Food and Drug Administration; FWHM, full width at half maximum; GC/MS, gas chromatography-mass spectrometry; HPLC, high performance liquid chromatography; IMS, ion mobility spectrometry; LOD, limit of detection; MS, mass spectrometry; ORA, Office of Regulatory Affairs; K_o, reduced mobility; MS/MS, tandem MS.

☆ *Disclaimer*: The findings and conclusions in this article have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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[1] The U.S. FDA has not published these results.

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organic compound and chemical warfare detection [21–27]. Using a portable ion mobility spectrometer as a 15 s screening tool, we obtained a limit of detection (LOD) of 2 ng of sibutramine hydrochloride (conversion to free base before analysis by IMS was unnecessary) in dietary supplements, which was 8 times lower than that obtained in a study using HPLC-UV [7]. HPLC-MS/MS has shown to be more sensitive than HPLC-UV for the detection of sibutramine by a factor as high as 1500 [5,7,9]. Although the LOD of sibutramine obtained using MS/MS was lower than that determined using the portable ion mobility spectrometer, products adulterated with just 0.1 mg of sibutramine can be detected using the portable spectrometer when a solvent extraction volume between 5 and 50 ml is used.

Portable devices are not meant to replace laboratory instruments; however, they can screen samples in order to effectively prioritize collection of samples to be sent to U.S. FDA laboratories for official testing. At the same time, using portable instruments will increase the frequency of testing products with the hope of eventually testing all products entering the U.S. To address this challenge, the Center for Drug Evaluation and Research Division of Pharmaceutical Analysis (DPA) with the assistance from the Office of Compliance and the Office of Regulatory Affairs (ORA) has developed high-throughput methods for portable spectroscopic instrumentation, requiring less than a minute per analysis [28]. In addition, the U.S. FDA has recently shown that IMS is applicable to the analysis of herbal dietary supplements suspected of containing erectile dysfunction (ED) drugs such as sildenafil, tadalfil and vardenafil as well as five synthetic analogues [13]. Using a benchtop IMS instrument to evaluate methanol extractions, the analysis time was 12 s and six of the ED drugs had a limit of detection of 0.2 ng. Like a benchtop instrument, the portable spectrometer used in our studies rapidly heats and desorbs the analyte from a substrate. One shortcoming of the portable system is that the maximum operating inlet and desorber temperatures are approximately 210 °C for the positive ion mode, which generally limits the analysis to drugs that can be volatilized near or below this temperature.

The purpose of the paper is to demonstrate the application of IMS to the rapid analysis of sibutramine in dietary supplements using a portable spectrometer. We evaluated five portable SABRE 4000 ion mobility spectrometers as tools to screen herbal dietary supplement extracts containing sibutramine. Following laboratory evaluation at DPA, the instruments were sent to five U.S. FDA laboratories around the U.S. for collaborative study. From the collaborative study results, we obtained the peak amplitude (intensity), the reduced mobility (K_0) and the full width at half maximum (FWHM) of the peak due to sibutramine from each ion mobility spectrum. The peak amplitudes were used to determine the critical level (CL) threshold and the LOD for the method and these values along with the other parameters were used to set the instrument thresholds for the detection of sibutramine. The potential for interference by caffeine was also investigated since dietary supplements often contain this compound.

2. Experimental

2.1. Materials

Sibutramine hydrochloride monohydrate was purchased from Sigma (St. Louis, MO). HPLC grade methanol, HPLC grade acetonitrile and glacial acetic acid were purchased from EMD Chemicals (Gibbstown, NJ). Caffeine and pyridoxine hydrochloride (vitamin B_6) were obtained from U.S. Pharmacopeia (Rockville, MD). An herbal dietary supplement for weight loss was purchased from a local store and spiked with sibutramine HCl monohydrate. According to the label claim of the dietary supplement product, each capsule contained 12.5 mg of vitamin B_6, 50 mg of magnesium (as oxide) and 570 mg of a proprietary blend, including approximately 97.5 mg of caffeine and caffeine-like stimulants. Cellulose acetate syringe filters (0.2-μm pore size, 17-mm membrane diameter) were purchased from Grace Davison Discovery Sciences (Deerfield, IL). The positive ion verification standard (chlorpheniramine) and sampling swabs (paper substrates) were purchased from Smiths Detection (Warren, NJ). Potassium chloride was purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Sibutramine reference standard and spiked dietary supplement sample preparation

Approximately 1 mg/ml of sibutramine HCl monohydrate was prepared in methanol. Using this solution, aliquots of 5, 10, 20, 30 and 40 μl were placed in 1.5 ml microcentrifuge tubes and vacuum dried (Thermo Electron Corp., SPD111 V SpeedVac concentrator and RVT4104 refrigerated vapor trap, Madison, WI) for approximately 45 min. The solution containing 1 mg/ml of sibutramine HCl monohydrate was diluted to 0.1 mg/ml of sibutramine HCl monohydrate. Aliquots of 10 and 20 μl of the diluted sibutramine solution were dispensed into 1.5 ml microcentrifuge tubes and vacuum dried for 45 min. A dietary supplement composite was prepared by transferring the contents of 20 capsules into an amber jar with a Teflon-lined cap and mixing with a turbula mixer (Chemical and Pharmaceutical Industry Co. Inc., model T1A, New York, NY) for 2 h. Approximately 3.5 mg of the mixed composite was added to each microcentrifuge tube that contained the dried aliquot of sibutramine (1, 2, 5, 10, 20, 30 and 40 μg). A blank sample was prepared by transferring 3.5 mg of the mixed composite into an empty microcentrifuge tube. A 20 μg sibutramine reference standard was prepared in a similar fashion (20 μl of 1 mg/ml sibutramine HCl monohydrate was dried in a microcentrifuge tube) except the composite was not added to the tube. These eight dietary supplement samples and the sibutramine reference standard were stored in a refrigerator below 10 °C until extracted into methanol. To prepare samples for analysis, 1 ml of methanol was added to each spiked sample, the sample was shaken for 30 s and filtered with a cellulose acetate syringe filter. The reference standard was treated in a similar manner, but was not filtered. It was not necessary to filter the standard solution since it did not contain particulates and was only used to ensure the instrument was working properly prior to the analysis of the dietary supplement extracts. A 1 μl aliquot of the resulting solution was dispensed on the sampling swab, allowed to dry and immediately analyzed using the SABRE 4000.

2.3. Analysis of caffeine and vitamin B_6

Typically, weight loss supplements contain a significant amount of caffeine and the presence of caffeine could affect the detection of sibutramine by IMS. Therefore, a study was conducted to see the effect of caffeine on the LOD of sibutramine. Prior to quantitation by HPLC, direct infusion positive ion electrospray ionization mass spectrometry (ESI-MS) was used to determine the amount of caffeine in the dietary supplement. The contents (approximately 700 mg) of one capsule was added to a 20 ml volumetric flask, diluted to volume with methanol, sonciated for 10 min, centrifuged for 10 min and filtered with a 0.2 μm cellulose acetate filter. From this solution, 0.1 ml was transferred to an HPLC vial and 0.9 ml of methanol was added. The diluted methanol extract of the dietary supplement was analyzed by direct infusion positive ion ESI-MS. Analysis by ESI-MS was performed with a Thermo Finnigan LCQ Deca XP ion trap mass spectrometer (San Jose, CA) using a sheath gas of 30 arb, spray voltage of 4 kV, capillary temperature of 300 °C and capillary voltage of 4 V.

For quantitation of caffeine in the dietary supplement by HPLC, approximately 10 mg of caffeine was transferred to a 20 ml volumetric flask and diluted to volume with methanol. From this caffeine standard solution, six dilution solutions were prepared at concentrations of 10, 50, 100, 200, 300 and 400 μg/ml of caffeine in methanol. To a 5 ml volumetric flask, 17.15 mg of dietary supplement composite was added and diluted to volume with methanol. Standard and dietary supplement solutions were filtered with a cellulose acetate filter prior to analysis by HPLC. HPLC was performed with an Agilent 1100 instrument (Santa Clara, CA) using a Waters SunFire C18 (2.1 mm × 150 mm 3.5 μm) column and a photodiode array detector at 274 nm to determine the concentration of caffeine in the dietary supplement capsules. The mobile phase was 0.5% acetic acid in 21% acetonitrile, the flow rate was 0.2 ml/min and the injection volume was 3 μl.

To investigate the influence of caffeine on the LOD of sibutramine, 10 solutions of caffeine (0.1–1 mg/ml) were prepared in methanol. A standard sibutramine solution of 2 ng/μl in methanol was prepared. An aliquot of the sibutramine solution was first spotted on the swab, allowed to dry, then 1 μl of the caffeine solution was spotted on the same location of the swab, allowed to dry and immediately analyzed using the portable ion mobility spectrometer. Triplicate analysis of each caffeine solution was performed using three instruments. The sibutramine solution without the presence of caffeine was also analyzed in triplicate with the same three spectrometers.

Standard solutions of 20 μg/ml of caffeine and vitamin B_6 were prepared separately in methanol. Each solution (1 μl) was analyzed using a benchtop ion mobility spectrometer, IONSCAN-LS.

2.4. Humidity effects on the detection of sibutramine

Water can influence ion drift times in IMS and since the instruments under investigation were intended for field use in uncontrolled environments, it was necessary to determine if humidity will affect the ion mobility of sibutramine. Four SABRE 4000 instruments were separately placed in a glove bag containing a solution of saturated potassium chloride in water, a stir plate and a hygrometer (Control Company Model 4410, Friendswood, Texas). When the relative humidity reached 80–90% inside the glovebag, 1 μl aliquots of sibutramine HCl in methanol (5, 10, 20, 30 and 40 ng/μl) were analyzed in triplicate. The analysis was repeated with a humidity of about 60%.

2.5. Ion mobility spectrometry

Five SABRE 4000 (Smiths Detection, Danbury, CT) portable ion mobility spectrometers (dimensions of 14.5″ × 4″ × 4.5″ and weight of 7 lbs) with Instrument Manager software version 5.057 (Smiths Detection, Danbury, CT) were used in this study. The preset narcotics control parameters (positive ion) and particle mode were used for the detection of sibutramine. Using a type K thermocouple (Control Company model 4131CC, Friendswood, Texas), the desorber temperature was measured for each IMS instrument using a programmed desorber temperature from 150 °C to 210 °C with 10 °C increments. A measured desorber temperature of approximately 190 °C was used for the analysis of sibutramine and the drift tube temperature was preset to approximately 130 °C for each instrument. The analysis time was 15 s with a scan period of 25 ms, a pulse width of 0.3 ms, 12 segments with 50 co-added scans per segment and a drift flow of 200 cc/min. The instruments were baked-out for 2 h at 180 °C and cooled for 2 h prior to the analysis of sibutramine in dietary supplements. After sample analysis, the instrument was purged with clean air as needed.

A benchtop ion mobility spectrometer, IONSCAN-LS (Smiths Detection, Danbury, CT) was used in positive ion mode to analyze solutions of caffeine and vitamin B_6. The instrument parameters included a drift tube temperature of 232 °C, an inlet temperature of 289 °C, a desorber temperature of 291 °C and a drift flow of 300 cc/min.

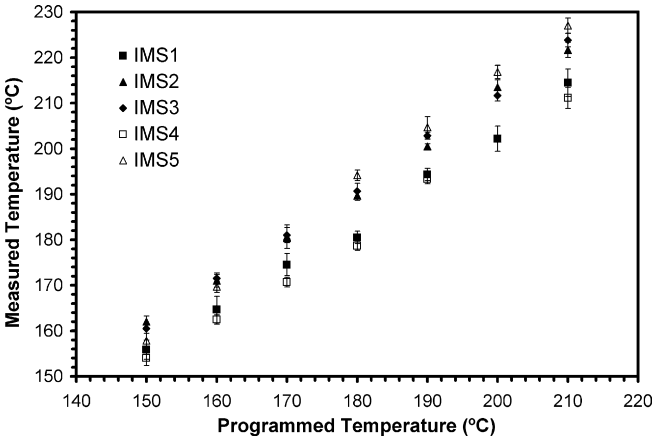

Fig. 1. Comparison of the desorber temperatures of the five SABRE 4000 IMS instruments.

3. Results and discussion

Prior to sending the portable SABRE 4000 IMS spectrometers to five U.S. FDA laboratories, the performance of these instruments was evaluated by comparing the desorber temperatures and the ion mobility spectra of sibutramine. In addition, we investigated the effect of humidity on the reduced mobility of sibutramine. The data obtained from this initial study were used to set the preliminary instrument alarm parameters for the detection of sibutramine.

The desorber temperature of the ion mobility spectrometer is one of the most important instrument parameters for analyte detection. The desorber rapidly heats the sampling swab, allowing the analyte to be thermally vaporized from the swab's surface. Thus, if the temperature is not adequate, then the analyte will not be desorbed from the surface. Furthermore, if the temperatures of two instruments are different, then the sensitivity may also differ. To compare the programmed desorber temperatures of the five instruments, the temperature was measured for each instrument with a thermocouple attached to an unused sample swab. Fig. 1 shows a linear relationship between the programmed and measured desorber temperatures. This result demonstrated that the set temperature is not necessarily accurate, but that the setting can be adjusted to achieve the desired desorber temperature as demonstrated by Fig. 1. The effective upper limit on the desorber temperature was roughly 210 °C. At settings above this temperature, the instruments became unstable. For the analysis of sibutramine, the instruments were programmed to have a measured desorber temperature of 190 °C. Although sibutramine HCl monohydrate has a melting point near 195 °C [29], there was no need to convert the salt to the free base (melting point of 52-57 °C) [30] in order to detect it using the SABRE 4000 instrument. Thermal properties such as melting point and heat of vaporization can be used to estimate the appropriate desorber temperature, but the final temperature selection is best done empirically.

In order to compare the ion mobility spectra of sibutramine, we analyzed five solutions of sibutramine HCl (5, 10, 20, 30 and 40 μg/ml) in triplicate using four of the spectrometers. The reduced mobility of the peak was obtained from the ion mobility spectrum of each sibutramine solution analyzed. The analysis of the sibutramine HCl standard using the portable IMS instrument yielded a single peak at 18.6 ms in the positive ion mobility spectrum as

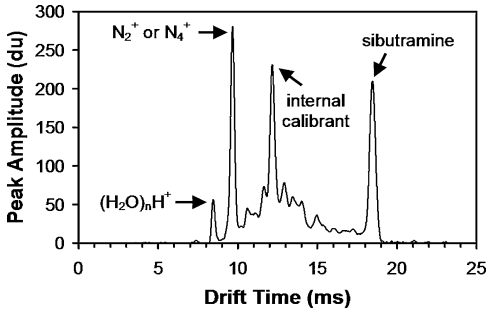

Fig. 2. Positive ion mobility spectrum of sibutramine HCl standard obtained using the SABRE 4000 instrument.

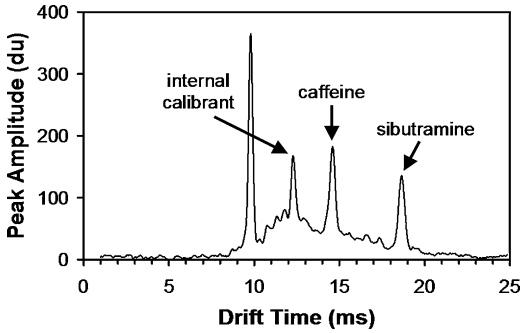

Fig. 3. Positive ion mobility spectrum of a dietary supplement sample containing sibutramine and caffeine acquired using the SABRE 4000 instrument.

demonstrated in Fig. 2. The peaks at 8.7 and 9.8 ms were most likely due to ions from water ($(H_2O)_nH^+$) and nitrogen (N_2^+ or N_4^+), respectively, and these peaks were also present in the absence of sibutramine. The peak at 12.3 ms was due to the instrument's positive ion internal calibrant, nicotinamide, which was used to calculate the reduced mobility of sibutramine. Averaged across four of the portable spectrometers, the reduced mobility of sibutramine was $1.1728 \pm 0.0010\ cm^2/(V\,s)$, which was used to program the instrument to detect sibutramine.

Environmental conditions and surroundings may lead to false detection. In order to see if we could simulate false negative and false positive results, we investigated the effects of humidity on the detection of sibutramine and tested three cleaning agents using the SABRE 4000 instruments, respectively. The instrument's internal calibrant is used to correct for small environmental changes in humidity, temperature and pressure. The instrument also contains a replaceable air purification cartridge used to remove much of the moisture from atmospheric air (serving as the drift gas) prior to it entering the instrument. As this cartridge becomes spent due to water build-up, the internal calibrant can shift out of its drift time detection range (the instrument is programmed with a calibrant drift time variability of 0.05 ms). To determine the influence of humidity on sibutramine's reduced mobility, we analyzed different levels of sibutramine with four SABRE 4000 instruments at both 60% and 80–90% relative humidity. We found that the relative humidity did not significantly change the reduced mobility of the sibutramine. At about 60% relative humidity (laboratory conditions), the reduced mobility of sibutramine was $1.1728 \pm 0.0010\ cm^2/(V\,s)$ and with a relative humidity of 80–90%, the reduced mobility slightly decreased to $1.1712 \pm 0.0007\ cm^2/(V\,s)$. This minimal difference of $0.0016\ cm^2/(V\,s)$ in reduced mobilities was used to establish the drift time variability of 0.05 ms for sibutramine. The change in humidity did not cause a false negative result when sibutramine was analyzed. However, we found that false detection of sibutramine occurred when chemical vapors from a cleaning agent were present in the laboratory. Fumes from floor wax adsorbed onto sampling swabs in less than 30 s, yielding erroneous false positive results for sibutramine. As a consequence, testing an unused swab before analyzing a sample was found to be critical in order to avoid errors from chemical vapors that may be present in a laboratory or manufacturing location.

Based on the preliminary data discussed above, each spectrometer's alarm was programmed for the detection of sibutramine. The instruments and samples were then shipped from the DPA laboratory to regional U.S. FDA laboratories in Jamaica NY, Philadelphia PA, Atlanta GA, Detroit MI and Irvine CA. In collaboration with these laboratories, dietary supplements containing sibutramine HCl were evaluated to determine the overall reduced mobility, FWHM, LOD and the 95% CL (used to set the peak amplitude threshold) for five SABRE 4000 portable ion mobility spectrometers. When the samples were analyzed, a typical ion mobility spectrum shown in Fig. 3 was obtained. Using the collaborative study data, it was determined that sibutramine (detected in the supplement extracts containing 2–40 ng/μl) had a reduced mobility of $1.1712 \pm 0.0015\ cm^2/(V\,s)$ and a FWHM of 424 ± 30 μs averaged across all five SABRE 4000 instruments. Fig. 4 shows the combined calibration plot from all data from the five instruments. The peak amplitudes of sibutramine were found to vary somewhat for triplicate analyses on individual instruments as well as across instruments. For example, when the dietary supplement extract containing 20 ng/μl of sibutramine was measured using a single instrument, the peak amplitude was 124 ± 34 du. Across all instruments the peak amplitude was 162 ± 72 du. Residual analyte, sample delivery, desorption and ionization efficiencies and detector response across instruments all contribute to variability. We believe that when higher amounts of sibutramine such as 20–40 ng were analyzed, variation in the ionization efficiency, space charge effects and precipitation onto the walls of the drift tube result in higher peak amplitude variability. The linear least-squares method was used to determine the relationship between the amount of sibutramine (C) and the amplitude of the sibutramine peak (S) in the ion mobility spectrum, and the result based on the data in Fig. 4 is given in Eq. (1)

$$S = mC + b \tag{1}$$

where the slope $m = 7.6229$ amplitude units per nanogram reflects the sensitivity of the instrument and the intercept $b = 6.4181$ du reflects the baseline signal. The LOD was determined in accordance with ICH Q2B guidance [31] as

$$\mathrm{LOD} = \frac{3\sigma_{\mathrm{bl}}}{m} \tag{2}$$

where σ_{bl} is the standard deviation of the blank signal across all instruments. From the collaborative study, the standard deviation of the blank signal across all instruments was found to be 4.3, and the resulting instrument LOD was 2 ng. LODs for individual instruments ranged from 1.2 to 2.5 ng. A typical capsule contained 770 mg of dietary supplement powder, and using the extraction and dilu-

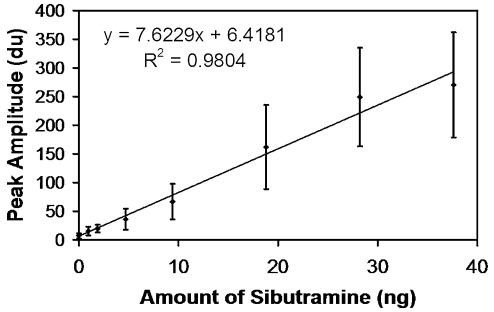

Fig. 4. Calibration plot from the analysis of sibutramine in dietary supplements using data from five SABRE 4000 instruments.

tion procedure described in section 2.2, the 2 ng LOD is equivalent to 0.44 mg of sibutramine in a typical capsule. Since our extraction method resulted in a dilution factor of about 220,000, at least 10-fold lower detection limits can be easily achieved by reducing the dilution factor. Because the amount of undeclared sibutramine in a dietary supplement is typically unknown, a guideline for preparing and analyzing samples is provided (see the 5-step procedure).

The overall critical level at 95% confidence was determined from the data collected by five U.S. FDA laboratories each using a different portable instrument. The critical level can be used as a pass/fail decision threshold and was defined as the concentration at which a blank sample will pass (no sibutramine will be detected) with probability determined by the desired confidence level. At 95% confidence and assuming that the peak amplitudes of the blank sample were normally distributed, the critical level of the signal amplitude (CL_{amp}) was determined according to Eq. (3), which was used to set the SABRE 4000 peak amplitude alarm threshold for sibutramine. From the collaborative study, the mean blank amplitude ($\bar{S}_{bl}$) was 7.0 du, σ_{bl} was 4.3 du as previsously stated and CL_{amp} was 14 du.

$$CL_{amp} = \bar{S}_{bl} + 1.645\sigma_{bl} \quad (3)$$

Eq. (4) converts the amplitude critical level to a mass (CL_{mass}) of sibutramine delivered to the instrument,

$$CL_{mass} = \frac{CL_{amp} - b}{m} \quad (4)$$

resulting in a critical level of 1 ng of sibutramine HCl, which was used to set the SABRE 4000 peak amplitude alarm threshold. Using this value in the alarm settings will result in a false positive alarm for 5% of blank samples. Assuming that low level sibutramine containing samples have the same distribution of measured values as the blank sample, the instrument LOD can also be estimated as $[(CL_{amp} + 1.65\sigma_{bl}) - b]/m = 2$ ng A sample containing sibutramine at this LOD will be detected 95% of the time. This alternative method of determining the LOD has the advantage of providing an LOD that is statistically unambiguous. From the collaborative study, the new alarm settings were $K_0 = 1.1712$, FWHM = 424 and peak amplitude threshold = 14 for the detection of sibutramine in dietary supplements (the drift time variability was kept at 0.05 ms).

Once the LOD of sibutramine was determined from the collaborative study, we investigated factors that could potentially increase the LOD. The detection of sibutramine in the presence of other compounds extracted from the herbal supplement was a concern due to ion suppression effects, resulting in decreased sensitivity. Hence, a compound with a higher ionization efficiency than sibutramine would most likely result in a reduced signal for sibutramine. Analysis by positive ion ESI-MS showed that a methanol extract of the dietary supplement contained caffeine, a common stimulant present in weight loss supplements, and vitamin B_6 (data not shown here). The analysis of a sibutramine-spiked dietary supplement extract using the SABRE 4000 afforded the ion mobility spectrum shown in Fig. 3. There was a dominant peak at 14.6 ms, which was due to caffeine, but vitamin B_6 was not detected. The amount of caffeine in each dietary supplement capsule determined by HPLC was approximately 100 mg, which was in agreement with the product warning label, stating that two capsules contain approximately 195 mg of caffeine or caffeine-like stimulants. In 3.5 mg of dietary supplement composite, the amount of caffeine present was 0.44 mg. Thus, when dissolved in 1 ml of methanol, the sample solution contained 440 ng/μl of caffeine. A 1 μl aliquot of this solution was analyzed using the portable IMS instrument. Hence, 440 ng of caffeine was delivered to the instrument each time the dietary supplement extract was analyzed. When the amount of caffeine was below 100 ng, it was not observed in the ion mobility spectrum using the SABRE 4000. To determine if caffeine influenced the LOD of sibutramine, we performed analyses with 2 ng of sibutramine in the presence of up to 1000 ng of caffeine and found no increase in the LOD as shown in Fig. 5. Vitamin B_6 was also present in the dietary supplement (12.5 mg/capsule). Therefore, the amount of vitamin B_6 in 3.5 mg of dietary supplement corresponded to approximately 60 ng. At this level, this vitamin was not observed in the ion mobility spectrum. Even at 800 ng of vitamin B_6, the ion mobility spectrometer was not able to detect the vitamin. The detection of caffeine over vitamin B_6 using the portable spectrometer was hindered by the desorber temperature rather than the ionization efficiency of the vitamin. The spectrometer utilized a thermal desorber temperature of 190 °C to vaporize the analyte from a paper substrate. Interestingly, the melting point of caffeine (238 °C) is above that of the vitamin (205–212 °C). [32] However, sublimation of caffeine occurs at 178 °C [32]. Although the ionization mechanisms of IMS and ESI are not identical, analysis of the dietary supplement by ESI-MS was used to compare the relative proton affinities of caffeine and vitamin B_6. A diluted dietary supplement methanol extract was analyzed by direct infusion positive ion ESI-MS (mass spectrum not shown). The amount of caffeine (0.5 mg/ml) in the methanol extract was 8-fold greater than that of vitamin B_6 (0.0625 mg/ml). The predominant MS signal at m/z 170 $[M+H]^+$ due to vitamin B_6 was approximately 20-fold more intense than the signal due to caffeine at m/z 195 $[M+H]^+$, suggesting the vitamin has a higher proton affinity. We then analyzed caffeine and vitamin B_6 standards using a benchtop IMS instrument with a desorber temperature of 291 °C. Analysis of 20 ng of vitamin B_6 yielded a signal with a peak intensity of about 1200 du whereas when the same amount of caffeine was analyzed, its signal was about 20-fold less intense (spectra not shown), confirming that the vitamin has a higher gas-phase proton affinity than that of caffeine. Therefore, the lack of signal of vitamin B_6 in the positive ion mobility spectrum acquired using the portable spectrometer was most likely due to an insufficient desorber temperature.

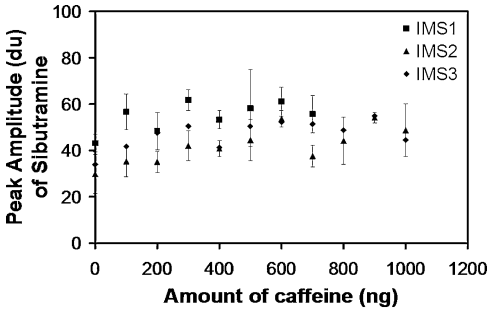

Fig. 5. Analysis of 2 ng of sibutramine in the presence of caffeine (100–1000 ng) using three different SABRE 4000 instruments.

Another important parameter to consider when developing a method using these portable IMS instruments is that the linear dynamic range is about one order of magnitude, so overloading the instrument with analyte is easy. Typically, persistent analytes can be removed from the instrument by purging the system with clean air for 20 s up to three times. However, the system may necessitate rigorous cleaning and/or replacing parts of the instrument if overloaded. Thus, when analyzing a compound for the first time, it is better to analyze a low amount (1–10 ng) of sample rather than a high amount such as 100 ng. From the collaborative study, when 30 ng and 40 ng of sibutramine were analyzed, saturation of the IMS chamber occurred and the instrument required more than three cleaning cycles to remove the drug. Hence, no more than 20 ng of sibutramine should be analyzed with the SABRE 4000. When the amount of adulterant in contaminated dietary supplements is unknown, proper dilution of the sample is essential when preparing the sample for analysis by IMS. Hence, the target sample concentration range was 2–20 ng/μl of sibutramine as determined from

the LOD and instrument saturation. Counterfeit weight loss products have contained 0.1–40 mg of sibutramine HCl/capsule. Based on these values, the following 5-step procedure can be used as a guide to prepare and analyze dietary supplements that may potentially contain sibutramine in order to prevent saturation of the IMS instrument:

1. Transfer the contents of one capsule or one ground tablet into a 20 ml volumetric flask, dilute with methanol and shake.
2. Filter 3 ml of this solution from step 1 using a syringe filter, preferably a 0.2 μm PTFE filter.
3. Dilute 0.1 ml of the filtered solution from step 2 using a 10 ml volumetric flask and methanol. Analyze 1 μl of the diluted solution.
4. If sibutramine was not detected in the solution prepared from step 3, then dilute 1 ml of the filtered solution from step 2 using a 10 ml volumetric flask and methanol. Analyze 1 μl of the diluted solution.
5. If sibutramine was not detected in the solution prepared from step 4, then analyze 1 μl of the filtered solution from step 2.

Replicate analysis of each solution is suggested. We also recommend analyzing a sibutramine reference standard prior to any sample analysis to ensure that the instrument is working properly.

4. Conclusion

We have demonstrated that portable IMS instruments can be applied to the analysis of low levels of sibutramine. Results obtained using five portable ion mobility spectrometers were used to aid in determination of instrumental parameters for the detection of sibutramine in dietary supplements. Among the five SABRE 4000 instruments, the LODs of sibutramine were consistent with an overall LOD of 2 ng of sibutramine HCl. Counterfeit herbal dietary supplement samples containing unknown amounts of sibutramine can be prepared using two dilution factors. Samples should be prepared to contain 2–20 ng/μl of sibutramine and these concentrations can be obtained using the suggested 5-step procedure. The sample preparation and the portable IMS instrument methods for the detection of sibutramine are simple enough that they can be used to examine products at ports of entry to prevent adulterated materials from entering the marketplace.

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References

[1] U.S. Food and Drug Administration, FDA warns consumers about "More Weight Loss Products Added to Consumer Alert", http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm103184.htm (Last accessed: August 24, 2010).
[2] D. Blachut, A. Siwinska-Ziolkowska, A. Kobylecka, Z. Czarnocki, Analysis the composition of Chinese "herbal" slimming aids, Z. Zagadnien. Nauk. Sadowych. 66 (2006) 199–211.
[3] D. Blachut, A. Siwinska-Ziolkowska, B. Szukalski, K. Wojtasiewicz, Z. Czarnocki, A. Kobylecka, M. Bykas-Strekowska, Identification of N-desmethylsibutramine as a new ingredient in Chinese Herbal dietary supplements, Z. Zagadnien. Nauk. Sadowych. 70 (2007) 225–235.
[4] R.D. Blackledge, P.D. Sorenson, The identification of sibutramine, Microgram 33 (2000) 18–20.
[5] M.J. Bogusz, H. Hassan, E. Ai-Enazi, Z. Ibrahim, M. Al-Tufail, Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies, J. Pharm. Biomed. Anal. 41 (2006) 554–564.
[6] Y. Chen, L. Zhao, F. Lu, Y. Yu, Y. Chai, Y. Wu, Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS, Food Addit. Contam. Part A-Chem. 26 (2009) 595–603.
[7] Z.Q. Huang, S. Xiao, D. Luo, B. Chen, S.Z. Yao, Simultaneous determination of sibutramine and N-di-desmethylsibutramine in dietary supplements for weight control by HPLC-ESI-MS, J. Chromatogr. Sci. 46 (2008) 707–711.
[8] J. Jung, M. Hermanns-Clausen, W. Weinmann, Anorectic sibutramine detected in a Chinese herbal drug for weight loss, Forensic Sci. Int. 161 (2006) 221–222.
[9] J. Wang, B. Chen, S. Yao, Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS, Food Addit. Contam. 25 (2008) 822–830.
[10] P. Zou, S.S.Y. Oh, K.H. Kiang, M.Y. Low, B.C. Bloodworth, Detection of sibutramine, its two metabxolites and one analogue in a herbal product for weight loss by liquid chromatography triple quadrupole mass spectrometry and time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 614–618.
[11] Screening for Sibutramine and Sibutramine Analogs in Weight Loss Supplements by Gas Chromatography-Mass Spectrometry, No 4455, U.S. Department of Health and Human Services, Food and Drug Administration.
[12] G.A. Eiceman, D.A. Blyth, D.B. Shoff, A.P. Snyder, Screening of solid commercial pharmaceuticals using ion mobility spectrometry, Anal. Chem. 62 (1990) 1374–1379.
[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] A.H. Lawrence, Ion mobility spectrometry mass-spectrometry of some prescription and illicit drugs, Anal. Chem. 58 (1986) 1269–1272.
[15] A.H. Lawrence, Characterization of benzodiazepine drugs by ion mobility spectrometry, Anal. Chem. 61 (1989) 343–349.
[16] R.M. O'Donnell, X.B. Sun, P.D. Harrington, Pharmaceutical applications of ion mobility spectrometry, Trac-Trends Anal. Chem. 27 (2008) 44–53.
[17] C. Qin, A. Granger, V. Papov, J. McCaffrey, D.L. Norwood, Quantitative determination of residual active pharmaceutical ingredients and intermediates on equipment surfaces by ion mobility spectrometry, J. Pharm. Biomed. Anal. 51 (2010) 107–113.
[18] M.A. Strege, Total residue analysis of swabs by ion mobility spectrometry, Anal. Chem. 81 (2009) 4576–4580.
[19] M.A. Strege, J. Kozerski, N. Juarbe, P. Mahoney, At-line quantitative ion mobility spectrometry for direct analysis of swabs for pharmaceutical manufacturing equipment cleaning verification, Anal. Chem. 80 (2008) 3040–3044.
[20] R. Fernandez-Maestre, H.H. Hill Jr., Ion mobility spectrometry for the rapid analysis of over-the-counter drugs and beverages, Int. J. Ion Mobility Spectrom. 12 (2009) 91–102.
[21] J.S. Babis, R.P. Sperline, A.K. Knight, D.A. Jones, C.A. Gresham, M.B. Denton, Performance evaluation of a miniature ion mobility spectrometer drift cell for application in hand-held explosives detection ion mobility spectrometers, Anal. Bioanal. Chem. 395 (2009) 411–419.
[22] X.Y. Liu, S. Nacson, A. Grigoriev, P. Lynds, J. Pawliszyn, A new thermal desorption solid-phase system for hand-held ion mobility microextraction spectrometry, Anal. Chim. Acta 559 (2006) 159–165.
[23] P. Rearden, P.B. Harrington, Rapid screening of precursor and degradation products of chemical warfare agents in soil by solid-phase microextraction ion mobility spectrometry (SPME-IMS), Anal. Chim. Acta 545 (2005) 13–20.
[24] J. Stach, T. Arthen-Engeland, J. Flachowsky, H. Borsdorf, A simple field method for determination of MTBE in water using hand held ion mobility (IMS), Int. J. Ion Mobility Spectrom. 5 (2002) 82–86.
[25] S. Zimmermann, S. Barth, W.K.M. Baether, J. Ringer, Miniaturized low-cost ion mobility spectrometer for fast detection of chemical warfare agents, Anal. Chem. 80 (2008) 6671–6676.
[26] C. Tiebe, H. Miessner, B. Koch, T. Hubert, Detection of microbial volatile organic compounds (MVOCs) by ion-mobility spectrometry, Anal. Bioanal. Chem. 395 (2009) 2313–2323.
[27] T. Keller, A. Keller, E. Tutsch-Bauer, F. Monticelli, Application of ion mobility spectrometry in cases of forensic interest, Forensic Sci. Int. 161 (2006) 130–140.
[28] J.F. Kauffman, C.M. Gryniewicz-Ruzicka, S. Arzhantsev, J.D. Dunn, J.A. Spencer, S. Wolfgang, X. Li, L.N. Pelster, B.J. Westenberger, L.F. Buhse, Pharmaceutical surveillance with rapid spectroscopic screening technologies, Am. Pharm. Rev. 13 (2010) 44–49.
[29] S. Budavari, The Merck Index: an encyclopedia of chemicals, drugs, and biologicals, Merck & Co., Inc., Whitehouse Station, NJ, 1996.
[30] D.R. Rao, R.N. Kankan, Sibutramine free base in crystalline form and its pharmaceutical use, WO2004/099119 (2004).
[31] Q2B Validation of Analytical Procedures: Methodology, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), U.S. Department of Health and Human Services, Food and Drug Administration, 1996.
[32] A.C. Moffat, Clarke's Analysis of Drugs and Poisons, 3rd ed., Pharmaceutical Press, London, 2004.