Ion mobility spectrometry for the rapid analysis of over-the-counter drugs and beverages

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Abstract In the pharmaceutical industry, there are increasing requirements for analytical methods in quality assessment for the production of drugs. In this investigation, ion mobility spectrometry (IMS) was used for the rapid qualitative separation and identification of active ingredients in generic over-the-counter drugs and food additives in beverages. The active ingredients determined in drugs were acetaminophen, aspartame, bisacodyl, caffeine, dextromethorphan, diphenhydramine, famotidine, glucosamine, guaifenesin, loratadine, niacin, phenylephrine, pyridoxine, thiamin, and tetrahydrozoline. Aspartame and caffeine were determined in beverages. Fourteen over-the-counter drugs and beverages were analyzed. Analysis times below 10 s were obtained for IMS, and reduced mobilities were reported for the first time for 12 compounds. A quadrupole mass spectrometer coupled to a mobility spectrometer was used to assure a correct peak assignment. The combination of fast analysis, low cost, and inexpensive maintenance of IMS instruments makes IMS an attractive technique for the qualitative determination of the active ingredients in over-the-counter drugs and food additives in manufacture quality control and cleaning verification for the drug and food industries.

Keywords Ion mobility spectrometry · Over-the-counter drug · Food additive · Sweetener

Introduction

The introduction of modified foods, as well as food, drug, and cosmetic additives has been a major change in people's lives over the past three decades. Food and beverages are being altered to make them more appetizing and colorful, to retard spoilage, and to conceal spoiled products. A public increasingly interested in healthy food and aware of the health implications of these additives, together with new revelations from the scientific community on their health effects, have driven food control institutions to issue regulations on the use of these chemicals.

There is controversy on the health risks of food additives (colorings, flavorings, and preservatives). Some authors claim that artificial sweeteners, such as saccharin, cyclamate, and aspartame, do not pose carcinogenic threats [1]. However, other studies show these sweeteners as a human cancer risk [2, 3]. In addition, it was demonstrated that some food additives produce allergic responses [4] and may increase hyperactivity in children with behavior problems [5, 6].

There is a growing need in the food and pharmaceutical industries for rapid, low-cost, and sensitive analytical methods in quality assessment and cleaning verification for the quality control and production of over-the-counter drugs and food [1]. A rapid, low-cost, sensitive method is required for routine screening of batches of raw materials, the production line, and the final commercial products to avoid deterioration and changes in specifications. Rapid cleaning verification is also a challenge related to screening pharmaceutical components on production equipment to avoid contamination of future products [7]. The techniques currently used for quality control in the pharmaceutical industry, such as HPLC and total organic carbon (TOC), are slow and relatively inaccurate [7, 8]. TOC is

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fast and simple, but it can produce false positive results because it finds all organic carbon and cannot distinguish between excipients or cleaning residues and the active ingredients. These false indications are nonexistent in IMS, which gives quantitative, selective results [7, 9]

HPLC is a selective and popular analytical method for the determination of over-the-counter drugs and food additives, but it is very expensive and the analysis times are between 1 and 20 min [10, 11]. In contrast, IMS can develop analysis run times of about 20–45 ms, and lab-made IMS instruments can be assembled at low cost. Besides, the cost of maintenance of IMS instruments is much lower than that of HPLC. With IMS, cost savings are realized in two areas: costs of consumables (solvents and column materials) needed in HPLC and cost savings found in the IMS's speed [7]. Other techniques such as gas chromatography (GC) also suffer several shortcomings, namely, lengthy analysis times and use of solvents. An advantage of IMS over HPLC or GC is the absence of analysis problems associated with faulty or dirty columns. Moreover, IMS instruments are easy to use, as demonstrated by their widespread employment in military and aviation security applications.

Ion mobility spectrometry (IMS), introduced by the end of the 60's by Cohen and Karasek [12], is an analytical technique that separates gas-phase ions at atmospheric pressure. IMS is a fast, low-cost, and sensitive method ideal for the detection of trace quantities of volatile organic and inorganic compounds. The main applications of early IMS instruments were the detection of illegal drugs [13], explosives [14], and chemical warfare agents [15]. The introduction of electrospray ionization–ion mobility spectrometry (ESI-IMS) in the late 1980's allowed the easy analyses of non-volatile and labile samples [16]. The combination of ion mobility spectrometers with mass spectrometers permitted analysis of metabolomes [17] and proteomes [18], study of protein-protein and noncovalent protein-ligand complexes [19], imaging of tissues [20], and separation of carbohydrate isomers [21] and mixtures of peptides [22].

In an ion mobility spectrometer, samples are ionized at atmospheric pressure, and an electric field drives the ions through a drift tube where collisions occur between the ions and neutral buffer gas molecules. Ionization commonly occurs by a radioactive, electrospray, or corona discharge source. After ionization, the ions are focused into a drift tube composed of a desolvation and a drift region. In the desolvation region, ions are stripped of solvent molecules with the help of a preheated counter-current of neutral gas (the buffer gas), and pulsed into the drift region by an ion gate. The ions are then accelerated through the drift region by the electric field, where they obtain a constant drift velocity. This constant velocity (v , cm s^{-1}), proportional to

the electric field (E , cm^2s^{-1}), results from the accelerating electric field and the retarding effect of random collisions with the gas [23]:

$$K = \frac{v}{E} = \frac{L^2}{V \cdot t_d} \quad (1)$$

where L is the length of the drift region in cm, V the total voltage drop in volts across the drift region, and t_d the time the ion spends traveling the distance L in seconds. Ion mobilities depend on temperature and pressure. These temperature and pressure effects are normalized to standard conditions to compare values of K in different laboratories through the use of the reduced mobility constant (K_0 , $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$):

$$K_0 = K \frac{P}{760} \frac{273}{T} \quad (2)$$

where P is the pressure in the drift region in Torr and T the buffer gas temperature in Kelvin [24]. K_0 values are constant for every compound in a specific buffer gas. A collection of reduced mobility values from ambient pressure ion mobility spectrometry was published in 1986 [25].

Over-the-counter drugs have been analyzed by IMS-MS using a handheld mobility spectrometer with a radioactive source by characterizing the vapors produced through warming the pharmaceutical solids in air. Acetaminophen, brompheniramine, chlorpheniramine, pseudoephedrine, phenylpropanolamine, acetyl salicylic acid, and caffeine were determined using this method [26]. Eckers et al. used IMS coupled to liquid chromatography/mass spectrometry to improve the separation of drug-related materials from excipients such as polyethylene glycols (PEGs) that make difficult the detection of trace level impurities in drugs [27].

Budimir et al. analyzed pharmaceutical formulations using atmospheric pressure ion mobility spectrometry combined with liquid chromatography and nano-electrospray ionization. A beta blocker (timolol), antidepressant (paroxetine), analgesic (paracetamol), and opiate (codeine) preparations were studied [28]. Kent et al. found IMS to be an ultra-fast alternative to HPLC for the validation of cleaning verification in the pharmaceutical industry. IMS exceeded all validation requirements for specificity, precision, linearity, LOQ/LOD, accuracy, stability, and speed. Using IMS, the sample analysis portion of the method validation was approximately eight times faster than for HPLC to determine residual diphenhydramine on stainless steel surfaces [29]. Weston et al. [30], using IMS-time-of-flight MS coupled with desorption electrospray ionization (DESI) sample introduction, analyzed several prescription and over-the-counter drugs (OTC) drugs including an antiseptic cream (chlorhexidine), Paracetamol (acetaminophen), Zantac (ranitidine), and a nicotine-

containing skin patch, among others. A review on pharmaceutical applications of ion mobility spectrometry [9] includes an ample listing of pharmaceutical compounds identified by IMS with reduced mobilities, ionic species, and methods used to identify the compounds. However, none of the over-the-counter drugs analyzed in the present work is reported in that review.

Atmospheric pressure electrospray ionization ion mobility spectrometry (ESI-IMS) may offer a detection method for the low cost, fast, and sensitive analysis of pharmaceutical formulations and foods due to its rapid monitoring and high-resolution potential. This method may be an alternative to slow and expensive methods such as chromatography or inaccurate methods such as TOC. Therefore, the specific objectives of this investigation were to establish if IMS had the capability for the fast and sensitive qualitative determination of active ingredients and additives in complex OTC drug formulations and beverages.

Experimental section

Instrument An electrospray-ionization atmospheric-pressure ion mobility spectrometer coupled to a quadrupole mass spectrometer (Fig. 1) was used in this work.

The IMS instrument was built at Washington State University, and a complete description and schematics can be found in previous publications [31]. The mobility spectrometer included an electrospray source and a drift tube. The drift tube consisted of a reaction (desolvation) region, an ion gate, and a drift region. Both desolvation and drift region had alternating stainless steel rings, separated by ceramic insulating rings. The metal rings were connected in series by high temperature resistors (Caddock

Electronics Inc., Riverside, CA, $\pm 1\%$). The resistors were $0.5\ \Omega$ for the desolvation region and $1\ \text{M}\Omega$ for the drift region. The drift and desolvation region were 25 and 7.5 cm long, respectively, with an I.D. of 50 mm. A $432\ \text{V cm}^{-1}$ electric field was created in the drift tube when 10,800 V were applied to the first ring [32].

The ion gate, which pulsed the ions into the drift region, was a Bradbury-Nielsen-type. It was made of two series of forty 75- μm parallel Alloy-46 stainless steel wires (California Fine Wire Co., Grove Beach, CA) 0.6 mm apart. The wires were biased to a potential, creating an orthogonal field relative to the drift field to stop the ions from passing into the drift tube. The closure potential was 40 V higher for one set of wires (positive wires) and 40 V lower for the other set (negative wires). Positive and negative wires were alternated in the gate. This closure voltage was removed for 100 μs so that a narrow pulse of ions entered the drift region. In the first ring of the desolvation region, there was an electrospray (ESI) target screen made out of 2-mm stainless steel mesh with a 0.5-cm round orifice in the center. To help desolvate the ions created by electrospray, hot N_2 gas was introduced as a countercurrent through a stainless-steel tube at the end of the drift tube at a flow rate of 0.9 L/min. The buffer gas was heated by passing it through a 2-m long stainless-steel tube wound inside a heated aluminum block (Fig. 1). The mobility spectrometer was operated at atmospheric pressure (690–710 Torr in Pullman, WA).

Regular parameters used to operate the mobility spectrometer were: reaction region length, 7.5 cm; drift tube length, 25.0 cm; voltage at the first ring, 12.12 kV; voltage at the gate, 10.80 kV; drift field, 432 V/cm; gate closure potential, $\pm 40\ \text{V}$; gate pulse width, 0.1 ms; scan time, 35 ms; pressure, 690–710 Torr; buffer gas, nitrogen; buffer gas temperature, $150 \pm 2^\circ\text{C}$; buffer gas flow, $0.9\ \text{l min}^{-1}$ (Table 1).

The mass spectrometer used in this investigation was an ABB Extrel (Pittsburgh, PA) 150-QC quadrupole (0–4,000 amu). The software, electronics, and detector of this instrument were recently upgraded. The electron multiplier detector signal of the mass spectrometer was amplified and sent to the data acquisition systems by a Keithley model 427 amplifier (Keithley Instruments, Cleveland, OH). The mass spectrometer was run by Merlin software (version 3.0, ABB Extrel, Pittsburgh, PA), which collected the mass spectral data. The IMS data was collected by custom-made LabView software (National Instruments, Austin, TX), which controlled the ion gate. The electronics for IMS data acquisition and gate control were built at WSU [33].

Spectra were acquired in IMS, SIM-IMS, and mass spectrometry modes. In SIM-IMS mode (single ion monitoring), the mass spectrometer voltages are set so that only

Fig. 1 Instrument. Sketch of the atmospheric pressure ion mobility-quadrupole mass spectrometer

Table 1 ESI-APIMS operating conditions summary

Parameter	Settings
Reaction region length	7.5 cm
Drift region length	25.0 cm
ESI voltage	15.6 kV
Voltage at first ring	12.12 kV
ESI flow	180 $\mu\text{l hr}^{-1}$
Drift tube potential	10.80 $\pm$ 0.01 kV
Gate closure potential	$\pm$ 40 V
Gate pulse width	0.1 ms
Scan time	35 ms
Buffer gas	Nitrogen
Buffer gas temperature	150 $\pm$ 2 °C
Buffer gas flow	900 ml min $^{-1}$
Pressure	690–700 Torr

ions of a given mass to charge ratio or a range of ions are detected. These settings avoid interference of other ions when determining a specific compound, and mobility spectra of a specific ion or ions are collected; in IMS mode, the mass spectrometer is operated in RF only mode so that ions are pulsed into the drift region, and enter the mass spectrometer, where they are all detected without scanning; the mobility spectrum of all ions is collected in this mode. In mass spectrometry mode, there is no pulsing of the ions; all ions continuously enter the mass spectrometer, and mass spectra are obtained; the ion mobility spectrometer is used as a desolvation region, especially when ESI is in use.

Materials and reagents Tetrabutylammonium (TBA) chloride, 2,4-dimethylpyridine (2,4-lutidine), and 2,6-di-tert-butyl pyridine (DTBP) (ACS reagent grade, $\geq$ 98% purity), purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI), were used as chemical standards. Several over-the-counter drugs and energetic beverages were selected based on their popularity and availability. The drugs were chosen among pain relievers, antihistamines, cough suppressants, nutritional supplements, laxative and antacid medication, and eye drops. The active ingredients determined in drugs were acetaminophen, aspartame, bisacodyl, caffeine, dextromethorphan, diphenhydramine, famotidine, glucosamine, guaifenesin, loratadine, niacin, phenylephrine, pyridoxine, thiamin, and tetrahydrozoline. Aspartame and caffeine were determined in beverages. The drugs and beverages were purchased from local stores.

Sample preparation and introduction Sample preparation was simple. Liquids and creams were mixed with the ESI solution, until a homogeneous mixture was obtained, and

diluted. Solid sample preparation consisted in dissolving the whole pill, including the coating; solid samples were placed in a glass vial and shaken overnight in the ESI solution (4.75 H₂O:4.75 methanol:0.5 acetic acid). Acetic acid was used to improve protonation. The clear supernatant was diluted to 0.5 mM in the case of samples with one active ingredient; in the case of samples with multiple active ingredients, the solutions were diluted until the less concentrated component reached a 10- μM concentration.

The chemical standards (TBA ions, 2,4-lutidine, and DTBP) were prepared in the ESI solvent at a concentration of 50 μM . Liquid samples or solvent (ESI solution) were injected using 250- μl syringes by electrospray ionization (ESI) at a flow rate of 3 $\mu\text{l min}^{-1}$ into 40-cm long, 100- μm ID capillaries. These capillaries were connected through stainless steel unions (Valco, Houston, TX) to 50- μm ID capillaries. The end of these capillaries was placed in the center of the target screen in the first ring of the mobility spectrometer. To produce positive ions, the metallic union received a high voltage of 15.6 kV with a 3.5 kV bias with respect to the first ring at the entrance of the mobility spectrometer.

Resolving power Resolving power is an indication of separation efficiency and can be calculated in IMS by:

$$R = t_d/w \quad (3)$$

where t_d is the drift time of the ion of interest and w is the temporal peak width measured at half-height [34]. Regardless of its resolving power, an instrument cannot separate two compounds with identical drift times. R values can be changed in IMS by operating at high electric fields, but with the undesirable effect of operating the mobility spectrometer in a region where mobility depends on voltage (where Eq. 1 is no longer valid).

Calibration and identification of analytes All analytes were detected as $\text{M}\cdot\text{H}^+$ ions, and were identified by mass spectrometry; their m/z ratio in mass spectrometry was compared to the molecular weight of their protonated molecules or clusters. Analytes were further identified by comparing their reduced mobilities with values reported in the literature. IMS peak identification was confirmed by SIM-IMS. The mobility scale was adjusted following the method of Eiceman et al. [35], who recommend correcting reduced mobilities by comparing with standards:

$$\frac{K_{0(\text{unknown})}}{K_{0(\text{standard})}} = \frac{t_{d(\text{standard})}}{t_{d(\text{unknown})}} \quad (4)$$

where K_0 is the reduced mobility in $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$ and t_d is the drift time in ms. This method accounts for errors in measuring instrumental parameters and eliminates the need

for performing these measurements. 2,4-lutidine and DTBP are common standards used to calibrate the reduced mobility scale [35].

Results and discussions

Figures 2, 3, 4, and 5 show spectra of the rapid analysis of over-the-counter drugs and beverages obtained by ion mobility spectrometry. These samples were electrosprayed directly into the ambient pressure mobility spectrometer and separated and detected within 9 s.

Single-ingredient over-the-counter drugs

Figure 2 shows the mass and mobility spectra of over-the-counter (OTC) drugs that comprise a single active ingredient. Figure 2.1 displays the spectra of the sample Claritin (Claritin®, Schering, Memphis, TN). The mobility spectrum of the sample Claritin in Fig. 2.1a was generated by operating the quadrupole mass spectrometer in IMS mode. In this mode, all the ions pass through the mass spectrometer and are detected by the ion multiplier; this mode of operation yields the ion mobility spectra of all the ions in the sample, the total ion mobility spectrum. Claritin is an antihistaminic medication for treating allergies, favored over other antihistaminic formulations by its non-sedating properties (<http://Encyclopedia Britannica>.

Accessed on May 12, 2009). The electrosprayed solution of this sample produced a simple ion mobility spectrum with only a single peak, which occurred at a drift time of 33.5 ms. The ion species present in the mobility spectrum corresponded to the protonated peak of the pharmaceutical loratadine ($C_{22}H_{23}ClN_2O_2$), the active ingredient of Claritin. A K_0 value of $1.04 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ was calculated for loratadine using Eq. 4. Other ions from the solvent occurred in the spectrum at drift times shorter than 20 ms (not shown). 250 mobility measurements were averaged to obtain every mobility spectrum, which represents an analysis time below 9 s. In Figure 2.1b, the mass spectrum of the sample Claritin produced a primary peak at m/z 383.9. This is the protonated molecule of loratadine, with a molecular weight of 382.9 g mol^{-1} . 500 scans were averaged to obtain the mass spectra presented in Figure 2.1b. Because loratadine is an amine, it has a strong response in positive mode IMS, producing a single product ion peak through proton transfer reactions with the ions produced from the electrospray process. To ensure that the mobility peak at 33.5 ms was the peak at mass 383.9, the mass spectrometer was operated in single ion monitoring mode (SIM) in which only the m/z 383.9 peak was monitored; more specifically, a range of $\pm 1 \text{ Da}$ was scanned around the MS peak (from 382.9 to 384.9). In SIM mode, the ion of interest is monitored without the interference of other ions, increasing the signal to noise ratio. When the mass spectrometer was operated in SIM mode, the ion mobility spectrum obtained looked similar to

Fig. 2 Mobility (a) and mass spectra (b) of single-ingredient drugs. 2.1 Spectra of an antihistaminic medication (Claritin®, Schering, Memphis, TN) showing the protonated peaks of loratadine (382.9 g mol^{-1}) at 33.5 ms and m/z 383.9. 2.2 Spectra of a joint nutritional supplement (Spring Valley®, Schiff, Salt Lake City, UT) showing the protonated peaks of glucosamine (179.2 g mol^{-1}) at 23.1 ms and m/z 180.2. 2.3 Spectra of eye drops (Eyelieve®, Orallabs, Memphis, TN) showing the protonated peaks of tetrahydrozoline (200.3 g mol^{-1}) at 24.5 ms and m/z 201.3; the peak at m/z 224.3 and 26 ms may be the sodiated adduct of tetrahydrozoline. Mobility peaks were identified by SIM-IMS; mobility and mass spectra were averaged 250 and 500 times, respectively

Fig. 3 Mobility (a) and mass spectra (b) of two-ingredient drugs. 3.1 Spectra of a migraine medication (Excedrin®, Novartis) showing the protonated peaks of acetaminophen (151.1 g mol^{-1}) at 21.0 ms and m/z 152.1 and caffeine (194.2 g mol^{-1}) at 23.3 ms and m/z 195.2; acetyl salicylic acid, another component of the drug, must be detected in the negative mode. 3.2 Spectra of a cough syrup (Tussin DM Assured) showing the protonated peaks of guaifenesin (198.2 g mol^{-1}) at 23.8 ms and m/z 199.2 and dextromethorphan (271.4 g mol^{-1}) at 29.3 ms and m/z 272.4; a possible water cluster of guaifenesin was visible at 25 ms and m/z 217.2 and a sodium adduct at 25.6 ms and m/z 222.2. Mobility peaks were identified by SIM-IMS; mobility and mass spectra were averaged 250 and 500 times, respectively

that shown in Figure 2.1a, indicating that the ion occurring at 33.5 ms was the protonated molecule of loratadine.

Figure 2.2 presents the spectra of a joint nutritional supplement (Spring Valley®, Schiff, Salt Lake City, UT), whose active ingredient is glucosamine. Glucosamine is an amino sugar, precursor in the biosynthesis of glycosylated proteins and lipids, commonly used as a treatment for osteoarthritis (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). Figure 2.2a shows the total ion mobility spectrum of the nutritional supplement. The spectrum, produced by operating the quadrupole mass spectrometer in IMS mode, shows four peaks at 23.1, 17.0, 15.6, and 14.2 ms. The peak at 23.1 ms is the protonated peak of glucosamine ($\text{C}_6\text{H}_{13}\text{NO}_5$), for which a K_0 value of $1.54 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ was calculated. The three peaks at lower drift times correspond to reactant ions or other components of the drug. The mass spectrum of the joint nutritional supplement in Fig. 2.2b displayed peaks at m/z 198.2, 180.2, 91.1, 55.1, and 37.1. The protonated peak of

glucosamine occurred at m/z 180.2 (molecular weight of glucosamine = 179.2 g mol^{-1}), and a peak of the water cluster of glucosamine occurred at m/z 198.2. The peaks at lower masses correspond to reactant ions; m/z 55.1 and 37.1 can be assigned to $(\text{H}_3\text{O})_3\text{H}^+$ and $(\text{H}_3\text{O})_2\text{H}^+$, respectively. The assignment of the peak at 23.1 ms to the protonated peak of glucosamine was done by operating the mass spectrometer in SIM mode and scanning m/z 180.2 ± 1 . In SIM mode, only the peak at 23.1 ms was obtained in the mobility spectrum. Also using SIM, the peak at 14.2 ms was assigned to $(\text{H}_3\text{O})_n\text{H}^+$. The joint supplement medication contained another active ingredient, chondroitin, which is a sulfated chain of a variable number (approximately 100) of alternating glucuronic acid and N-acetylgalactosamine sugars. This component, important in the structure of cartilage, could not be detected because it comprises many chains of different lengths and molecular weights.

Figure 2.3 includes the mobility and mass spectra of a sample of eye drops (Eyelieve®, Orallabs, Memphis, TN). The active ingredient of Eyelieve is tetrahydrozoline, which constricts the conjunctival blood vessels to relieve the redness of the eye caused by minor ocular irritants. Tetrahydrozoline (Fig. 2.3a) produced peaks in the mobility spectrum at 24.5 ms and 25.8 ms. In the mass spectrum in Figure 2.3b, the protonated peak of tetrahydrozoline ($\text{C}_{13}\text{H}_{16}\text{N}_2$, molecular weight = 200.3 g mol^{-1}) occurred at m/z 201.3; other peak at m/z 224.3 can be assigned to the sodiated adduct of tetrahydrozoline; additional peaks at m/z 91.1, 73.1, 60.6, 37.1, and other small peaks below m/z 150 can be assigned to the reactant ions; these peaks are not shown in the mobility spectrum because they occur at drift times below 20 ms. Operating the mass spectrometer in SIM mode by selecting the peak at m/z 201.3, the peak at 24.5 was obtained, for which this mobility peak was assigned to the protonated peak of tetrahydrozoline. A K_0 value of $1.42 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ was calculated for this peak. The mobility peak at 25.8 ms was assigned to the sodiated adduct of tetrahydrozoline at m/z 224.3 using SIM mode.

A generic laxative medication (Women's Laxative, Rite Aid, Harrisburg, PA) and a generic antacid medication (Acid Reducer, Rite Aid, Harrisburg, PA) were also analyzed (spectra not shown). The active ingredient of the laxative medication was bisacodyl ($\text{C}_{22}\text{H}_{19}\text{NO}_4$, molecular weight = 361.4 g mol^{-1}), which is typically prescribed for relief of constipation and for management of neurogenic bowel dysfunction. The laxative medication sample yielded a protonated peak in the mass spectrum at m/z 362.4. Selecting this m/z in SIM mode, a peak at 35.4 ms was obtained for the protonated peak of bisacodyl, corresponding to a K_0 value of $0.98 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. Famotidine ($\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$, 337.4 g mol^{-1}), the active ingredient of the antacid

Fig. 4 Mobility (a) and mass spectra (b) of three-ingredient drugs. This figure demonstrates the rapid IMS separation and identification of complex formulations. 4.1 Spectra of an allergy and sinus headache medication (Benadryl®, Pfizer, Morris Plains, NJ) showing the protonated peaks of acetaminophen (151.1 g mol^{-1}) at 21.6 ms and m/z 152.1, phenylephrine (167.2 g mol^{-1}) at 22.6 ms and m/z 168.2, and diphenhydramine (255.4 g mol^{-1}) at 27.9 ms and m/z 256.4. 4.2 Spectra of a vitamin B supplement (People's Choice, Mason Vitamins, Miami Lakes, FL) showing the protonated peaks of niacin (123.1 g

mol^{-1}) at 19.9 ms and m/z 124.1, pyridoxine (169.2 g mol^{-1}) at 22.1 ms and m/z 170.2, and thiamin (265.4 g mol^{-1}) at 28.3 ms and m/z 266.4. 4.3 Spectra of a cold medication (Rite Aid, Camp Hill, PA) showing the protonated peaks of acetaminophen at 21.0 ms and m/z 152.1, phenylephrine at 23.1 ms and m/z 168.2, and dextromethorphan (271.4 g mol^{-1}) at 28.5 ms and m/z 272.4. Mobility peaks were identified by SIM-IMS; mobility and mass spectra were averaged 250 and 500 times, respectively

medication, is a histamine that inhibits stomach acid production, and it is commonly used in the treatment of gastric ulcer (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). Famotidine produced a strong mass peak at m/z 338.4. In the IMS mode, a single peak for the antacid medication occurred at 30.7 ms; this mobility peak was identified by SIM-IMS as the protonated peak of famotidine, and a K_0 value of $1.15 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ was calculated for it.

Two-ingredient OTC drugs

Figure 3 shows the mass and mobility spectra of common OTC drugs composed of two-ingredients. Figure 3.1 includes the mobility and mass spectra of a migraine

medication (Excedrin®, Novartis) containing the active components caffeine and acetaminophen. Caffeine is an alkaloid that acts as a psychoactive stimulant drug and a mild diuretic; acetaminophen is a widely used over-the-counter analgesic and antipyretic, and is a major ingredient in numerous cold and flu remedies (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). The mobility spectrum for the migraine medication produced two strong peaks at 23.3 ms and at 21.0 ms (Fig. 3.1a). SIM-IMS analysis allowed the identification of the peaks as the product ions of caffeine and acetaminophen, for which respective K_0 values of 1.66 and $1.53 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ were calculated. The mass spectrum in Fig. 3.1b shows the protonated peaks of caffeine ($\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$, 194.2 g mol^{-1}) and acetaminophen ($\text{C}_8\text{H}_9\text{NO}_2$, 151.1 g mol^{-1}) occurring at

Fig. 5 Mobility (a) and mass spectra (b) of energetic beverages and standard solutions. 5.1 Spectra of Diet coke showing the protonated peaks of caffeine (194.2 g mol^{-1}) at 23.2 ms and m/z 195.2 and aspartame (294.3 g mol^{-1}) at 29.5 ms and m/z 295.3. 5.2 Standard solution of caffeine and aspartame ($500 \mu\text{M}$) showing the protonated peaks of caffeine at 23.0 ms and m/z 195.2 and aspartame at 29.4 ms and m/z 295.3. The peaks at m/z 213.2 and 313.3 are most probably water clusters. 5.3 Spectra of an antioxidant water (Snapple®) showing the protonated peaks of niacin (123.1 g mol^{-1}) at 19.8 ms and 124.1 and caffeine at 23.0 ms and m/z 195.2. Mobility peaks were identified by SIM-IMS; mobility and mass spectra were averaged 250 and 500 times, respectively

m/z 195.2 and 152.1, respectively. Acetyl salicylic acid, another component of the drug, must be detected in the negative mode, which was not set up in our instrument at the time the experiments were performed.

The mobility and mass spectra of a cough syrup (Tussin DM Assured) are presented in Fig. 3.2. Guaifenesin and dextromethorphan were the active ingredients of the drug. Guaifenesin ($\text{C}_{10}\text{H}_{14}\text{O}_4$, 198.2 g mol^{-1}) is an expectorant drug used also in the treatment of asthma, fibromyalgia, and gout, and as analgesic; dextromethorphan ($\text{C}_{18}\text{H}_{25}\text{NO}$, 271.4 g mol^{-1}) is an antitussive drug, also used as a pain reliever (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). The mobility spectrum in Fig. 3.2a shows peaks at 23.8 ms (protonated peak of guaifenesin), 25.0 ms (water cluster of guaifenesin), 25.6 ms (sodium adduct of guaifenesin), and 29.3 ms (protonated peak of dextromethorphan). Guaifenesin and dextromethorphan occurred at m/z 199.2 and 272.4, respectively, in the mass spectrum (Fig. 3.2b). Two additional peaks were observed in the mass spectrum corresponding to the water cluster of guaifenesin at m/z 217.2 and its sodium adduct at m/z 222.2. K_0 values of 1.51 and $1.22 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ were obtained for guaifenesin and dextromethorphan, respectively.

Three-ingredient OTC drugs

Figure 4 illustrates the mobility and mass spectra of OTC drugs containing three-ingredients, demonstrating the rapid IMS separation and identification of complex over-the-counter (OTC) formulations. The spectra of a generic allergy and sinus headache medication (Benadryl®, Pfizer, Morris Plains, NJ) composed of acetaminophen, phenylephrine, and diphenhydramine is shown in Fig. 4.1. Phenylephrine, now the most common OTC decongestant in the United States, relieve swelling of the nasal mucosa accompanying such conditions as the common cold and hay fever; and diphenhydramine is a synthetic antihistamine used in the treatment of various conditions including hay fever, acute skin reactions (such as hives), contact dermatitis (such as from poison ivy), and motion sickness (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). The mobility peaks of the active ingredients of this drug, assigned by operating the instrument in SIM-ESI mode, occurred at 21.6 ms (acetaminophen), 22.6 ms (phenylephrine), and 27.9 ms (diphenhydramine) (Fig. 4.1a). The protonated peaks of acetaminophen (151.1 g mol^{-1}), phenylephrine ($\text{C}_9\text{H}_{13}\text{NO}_2$, 167.2 g mol^{-1}), and diphenhydra-

mine ($\text{C}_{17}\text{H}_{21}\text{NO}$, 255.4 g mol^{-1}), the strongest peaks in the mass spectrum, are shown in Fig. 4.1b; these peaks occurred at m/z 152.1, 168.2, and 256.4, respectively. K_0 values calculated for these compounds were: 1.66 (acetaminophen), 1.53 (phenylephrine), and $1.23 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ (diphenhydramine). Benadryl® contained 16 inactive ingredients, comprising waxes, tar dyes, polymers, and carbohydrates, which gave the mobility spectrum a noisy appearance.

Figure 4.2 illustrates the mobility and mass spectra of a generic vitamin B supplement (People's Choice, Mason Vitamins, Miami Lakes, FL). The spectra included the protonated peaks of niacin ($\text{C}_6\text{H}_5\text{NO}_2$, 123.1 g mol^{-1}), pyridoxine ($\text{C}_8\text{H}_{11}\text{NO}_3$, 169.2 g mol^{-1}), and thiamin ($\text{C}_{12}\text{H}_{17}\text{N}_4\text{OS}^+$, 265.4 g mol^{-1}). Niacin (vitamin B3 or nicotinic acid) prevents the deficiency disease pellagra; pyridoxine (vitamin B6) assists amino acid metabolism, as well as promoting red blood cell production; and thiamin (thiamine or vitamin B1), is necessary for carbohydrate metabolism in both plants and animal (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). The mobility spectrum of the vitamin supplement in Fig. 4.2a shows the protonated peaks of niacin (19.9 ms), pyridoxine (22.1 ms), and thiamin (28.3 ms). These peaks were assigned operating the instrument in SIM-ESI mode. The mass spectrum of the supplement is displayed in Fig. 4.2b, and included the protonated peaks of niacin (m/z 124.1), pyridoxine (m/z 170.2), and thiamin (m/z 266.4). Riboflavin and folic acid, other B vitamins present in the supplement, were not seen in the mass spectrum perhaps due to their low concentration. K_0 values calculated for these compounds were: 1.80 (niacin), 1.62 (pyridoxine), and $1.26 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ (thiamin).

The mobility and mass data generated in the analysis of a generic cold medication (Rite Aid, Camp Hill, PA) is presented in Fig. 4.3. This medication contained acetaminophen, phenylephrine, and dextromethorphan. Dextromethorphan is an antitussive drug and has been used also for pain relief (<http://Encyclopedia Britannica>. Accessed on May 12, 2009). The mobility spectrum of the cold medication (Fig. 4.3a) shows the protonated peaks of acetaminophen, phenylephrine, and dextromethorphan occurring at 21.0 ms, 23.1 ms, and 28.5 ms, respectively. SIM-IMS was used to identify the peaks. In the mass spectrum, the protonated peaks of the active ingredients occurred at m/z 152.1 (acetaminophen), m/z 168.2 (phenylephrine), and m/z 272.4 (dextromethorphan) in the cold medication (Fig. 4.3b). K_0 values calculated for these pharmaceuticals were: 1.66 (acetaminophen), 1.53 (phenylephrine), and $1.22 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ (dextromethorphan).

In general, the active ingredients in OTC drugs for all the medications analyzed had one or more heteroatoms,

such as nitrogen and oxygen, which imparted a large proton affinity to these ingredients. This high proton affinity allowed the active ingredients of OTC drugs to be ionized largely by proton transfer reactions with reactant ions in the electrospray process. This efficient ionization of the active ingredients produced clean mobility and mass spectra and sharp identifiable peaks with few exceptions. In all samples analyzed, the active ingredients and their hydrated or sodiated clusters produced the strongest peaks in the mass spectrum. All active ingredients were identified in the mobility spectra by SIM-IMS in spite of the complexity of the formulation, with few exceptions.

Analyses of beverages

The soda drink and bottled water industry in the US includes about 3,000 companies that manufacture and distribute beverages, with combined annual US revenue of \$70 billion (<http://www.scribd.com>. Accessed on May 12, 2009). The large production of beverages requires rapid and low-cost technologies for their chemical analysis. Figure 5 shows the mass and mobility spectra of energetic beverages and standard solutions of caffeine and aspartame. Caffeine (194.2 g mol^{-1}) and aspartame ($\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$, 294.3 g mol^{-1}) were determined in Diet coke and an antioxidant water. Aspartame is an artificial, non-saccharide sweetener, 180 times sweeter than sugar, without its high energy value. The IMS protonated peaks of caffeine and aspartame in Diet coke are shown in Fig. 5.1a; caffeine occurred at 23.2 ms and aspartame at 29.5 ms, as single peaks in the mobility spectrum. The peaks were assigned operating the instrument in SIM-IMS mode. Other peaks occurred at higher mobilities, probably the reactant ions and ions from other components in the beverage. The mass spectrum of Diet Coke is presented in Fig. 5.1b, showing the protonated peaks of caffeine at m/z 195.2 and aspartame at m/z 295.3. The baseline increased toward higher mobilities in the IMS spectrum and toward lower masses in the mass spectrum, probably due to the complexity of the sample, Diet Coke, which is a blend of colors, flavors, and other components.

Figure 5.2 presents the mobility and mass spectra of a standard solution of caffeine and aspartame. The mobility spectrum in Fig. 5.2a included the protonated peaks of caffeine at 23.0 ms and aspartame at 29.4 ms in a 500- μM standard solution, identified operating the instrument in the SIM-IMS mode. Figure 5.2b presents the mass spectrum of the standard solution showing the protonated peaks of caffeine at m/z 195.2 and aspartame at m/z 295.3. The peaks at m/z 213.2 and 313.3 were probably water clusters of caffeine and aspartame. These peaks can also be seen, although less clearly, in Fig. 5.1b.

Figure 5.3 illustrates spectroscopic data for the analysis of a sample of antioxidant water (Snapple®). In Fig. 5.3a, the protonated peaks of niacin (123.1 g mol^{-1}) and caffeine, identified by SIM-IMS, occurred at 19.8 ms at 23.0 ms, respectively, in the mobility spectrum of the beverage. The mass spectrum of this water in Fig. 5.3b produced the protonated peaks of niacin (123.1 g mol^{-1}) at m/z 124.1 and caffeine at m/z 195.2. Peaks of unknown ingredients occurred at m/z 219.3 and 141.1 in the mass spectrum and at 22.0, 21.5, and 21.1 ms in the mobility spectrum. K_0 values calculated in both beverages and the standards were 1.53 for caffeine and $1.16 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for aspartame.

These spectra revealed the capabilities of IMS for the rapid separation and identification of active ingredients in complex over-the-counter drugs and food additives in beverages. Some of the OTC drugs and beverages contained up to 18 ingredients several of which were complex mixtures of lipids, polymers, and carbohydrates. Moreover, the concentrations of the ingredients determined were dissimilar with differences up to approximately 500-fold in some cases. However, the spectra

obtained in most cases were clean, and the ingredients were identified easily. This IMS selectivity is based on the high proton affinity of active ingredients of OTC drugs and food additives due to their nitrogen and oxygen content. In the analysis of three-ingredients drugs, brompheniramine and chlorpheniramine could not be detected in the mobility spectra of two products probably due to ionization competition of other active ingredients present at higher concentrations. However, the protonated peaks of these two compounds were visible in the mass spectrum, although with a low intensity.

A common method to validate results in IMS is through comparison of reduced mobilities with literature values. Reduced mobilities of active ingredients in OTC drugs and food additives and their concentrations in the solution analyzed are presented in Table 2. Three reduced mobilities of active ingredients were found in the literature out of 15 ingredients determined: 1.67 (acetaminophen), 1.54 (caffeine), and $1.55 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (glucosamine). The reduced mobilities for these compounds obtained in this work were: 1.66 (acetaminophen), 1.53 (caffeine), and $1.54 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (glucosamine); these reduced mobilities

Table 2 Reduced mobilities of active ingredients in OTC drugs and food additives and their concentrations in the solution analyzed

Compound	$K_0, \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$		Concentration, mM
	This work	Literature values	
Acetaminophen	1.66	1.67 ^a	(1) 44, (3) 4.8, (4) 33
Aspartame	1.16		(12) 0.12
Bisacodyl	0.98		(7) 0.5
Caffeine	1.53	1.54 ^a 1.54 ^b	(4) 0.067, (12) 0.067, (13) 0.05, (14) 0.05
Dextromethorphan	1.22		(3) 0.058, (5) 0.057
Diphenhydramine	1.23		(1) 0.87, (6) 0.017
Famotidine	1.15		(11) 0.50
Glucosamine	1.54	1.55 ^c 1.37 ^d	(8) 0.5
Guaifenesin	1.51		(5) 1.0
Loratadine	1.04		(9) 0.5
Niacin	1.80		(2)
Phenylephrine	1.53		(1) 0.50, (3) 0.050
Pyridoxine	1.62		(2)
Tetrahydrozoline	1.42		(10) 0.05
Thiamin	1.26		(2)

^a [36]; ^b [37]; ^c [38]; ^d [39]; (1) Allergy and sinus headache medication (Benadryl®, Pfizer, Morris Plains, NJ). (2) Vitamin B supplement (People's Choice, Mason Vitamins, Miami Lakes, FL). (3) Cold medication (Rite Aid, Camp Hill, PA). (4) Migraine medication (Excedrin®, Novartis). (5) Cough syrup (Tussin DM Assured). (6) Allergy drug (Allergy liquid, Assured, Bio-Pharm, Levittown, PA). (7) Laxative medication (Women's Laxative, Rite Aid, Harrisburg, PA). (8) Joint nutritional supplement (Spring Valley®, Schiff, Salt Lake City, UT). (9) Antihistaminic medication (Claritin®, Schering, Memphis, TN). (10) Eye drops (Eyelieve®, Orallabs, Memphis, TN). (11) Antacid medication (Acid Reducer, Rite Aid, Harrisburg, PA); (12) Diet coke. (13) Antioxidant water (Snapple®). (14) Orange Twist (Eating Right®). The reproducibility of the values in this table was <2%, calculated as the relative standard deviation (RSD) of the reduced mobilities of five different samples analyzed on different days. The repeatability of the values in this table was <0.5%, calculated as the RSD of the reduced mobilities of five or more consecutive analyses (250 averages each) of the sample

showed only a 0.6% difference with respect to the literature values, which was within the maximum 2% of accepted variance for data obtained in different laboratories. All other active ingredients in Table 2 were first-time determined by IMS, or their reduced mobilities were not reported when they were determined. A mass-mobility correlation coefficient of -0.97 was found for the ingredients in Table 2, which indicates the increasing size, and decreasing mobility, as the molecular weight increased.

Resolving power and signal to noise ratio

A high resolving power is a desirable feature of analytical techniques intended to separate complex OTC drugs comprising several active and inactive ingredients. A high resolving power produces sharp analyte peaks that can be easily distinguished from peaks of other ingredients in the mobility spectra. Figure 6 demonstrates the high resolving powers obtained in SIM-IMS mode when analyzing OTC drugs and beverages. Resolving powers up to 140 were obtained with an average of 100. Figure 6 shows these high resolving powers in the mobility spectra of diphenhydramine in Benadryl® (resolving power 140), aspartame in

Diet Coke® (resolving power 126), and tetrahydrozoline in Eyelieve® (resolving power 113). These high resolving powers allowed the resolution of the complex mixtures analyzed in this work. The signal to noise ratio for these analysis were 61 for diphenhydramine in Benadryl®, 41 for aspartame in Diet Coke®, and 20 for tetrahydrozoline in Eyelieve® at concentrations of 0.87, 0.12, and 0.05 mM, respectively.

Analysis time

A short analysis time is another desirable feature of analytical techniques that increases production in the pharmaceutical and food industries due to rapid cleaning verification and quality control and lower labor requirements. A short analysis time contributes to reduce costs and increase competitiveness. The number of averages used to obtain mobility spectra in this investigation was 250; with a scan time of 35–40 ms, instrumental analysis times below 10 s were obtained. The short duration of the analyses represents one of the features that translate into economy in IMS. These short analysis times were lower than those for HPLC (not including sample preparation). HPLC is a common analytical technique for the analysis of over-the counter-drugs and determination of food additives, but the analysis times are between 1 and 20 min, and instruments and maintenance are expensive.

Conclusions

We demonstrated the fast qualitative determination of active ingredients in over-the-counter drugs and food additives in beverages without sample pretreatment using electrospray-ion mobility spectrometry (ESI-IMS). Analysis times below 10 s were obtained for these samples. Clear separation and identification of the analyte peaks were obtained after a simple dissolution step in composite samples with 10–19 declared ingredients, some of which were complex mixtures of waxes, carbohydrates, polymers, dyes, and other large molecules. Complex samples with analyte concentrations down to 17 μM were analyzed, with other components at higher concentrations, indicating high selectivity and sensitivity. Resolving powers up to 140, with an average of 100, allowed resolution of the complex mixtures analyzed in this work. The combination of fast detection times, selectivity, sensitivity, low cost, and easy maintenance of ESI-IMS instruments makes this technique an attractive alternative for the qualitative analysis of over-the-counter drugs and beverages.

Fig. 6 Resolving power. SIM-IMS spectra of drugs and food additives showing the high resolving powers obtained by IMS. **a** Diphenhydramine (Resolving power 140) in the allergy and sinus headache medication (Benadryl®, Pfizer, Morris Plains, NJ). **b** Aspartame (Resolving power 126) in Diet Coke. **c** Tetrahydrozoline (Resolving power 113) in eye drops (Eyelieve®, Orallabs, Memphis, TN). Mobility spectra were averaged 250 times

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