Development of Thread Spray Mass Spectrometry for Direct Analysis of Capsaicinoids in Complex Pepper Products

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Sierra Jackson

Graduate Program in Chemistry

The Ohio State University

2017

Master's Examination Committee:

Dr. Abraham Badu-Tawiah, Advisor

Dr. Anne Co

Abstract

Thread spray ambient ionization is described that uses single threads as a medium for complex mixture sampling and ionization. This novel approach was developed and demonstrated through the detection of various capsaicinoids from the interior of pepper fruits without destruction of the sample. Pepper residues present on the thread were analyzed by the application of DC voltage and solvent to cause field-induced charged droplet generation. Capsaicinoids extracted from the sample are contained in the electrosprayed droplets and transported to the mass spectrometer for characterization. The thread spray mass spectrometry method was optimized using commercially available materials like 100% cotton, cotton/polyester (35:65), 100% polyester, and nylon fabrics and subsequent applied for in-situ analysis of seven different pepper fruits and pepper spray residues on fabrics. The results indicated that the special physico-chemical characteristics of threads allowed a rapid and convenient sampling and ionization of pepper products for analysis by mass spectrometry. The thread spray experiment presented in this thesis is similar to that of other ambient substrate-based ionization methods in that there is minimal or no sample preparation required for analysis. Some advantages exist for thread spray ionization when compared with other substrate-based ambient ionization methods: (i) it is capable of ionizing analytes present in solution or previously deposited onto a fabric,

presenting a unique opportunity to directly analyze pepper spray residues on clothing for forensic applications; (ii) unlike metal substrates, the porous organic threads can provide selective absorption of components in a mixture and allow in-situ separation during analysis; (iii) various off-the-shelf thread types are available as substrate for analysis and will create a large degree of flexibility in experimental design; (iv) surface derivatization of thread surface is possible, which will enable the immobilization of various functional groups for enhanced analytical performance.

Dedication

For my family and friends

Acknowledgments

Thank you to Dr. Abraham Badu-Tawiah for accepting me into his group and providing valuable feedback and assistance when I needed it. Also to the Badu-Tawiah group and to Patricia Capone and Devin Swiner especially for helping me in lab.

Vita

May 2010	LaGrange Senior High School
May 2015	B.S. Chemistry, Louisiana State University
2015 to present	Graduate Teaching Associate, Department of Chemistry, The Ohio State University

Fields of Study

Major Field: Chemistry

Table of Contents

List of Tables

List of Figures

List of Schemes

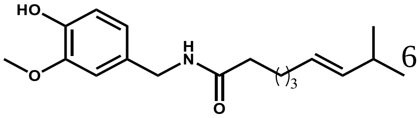
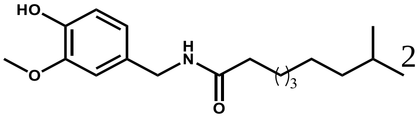
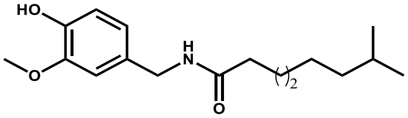
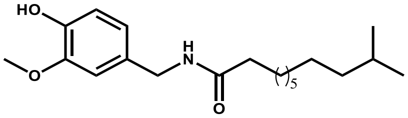
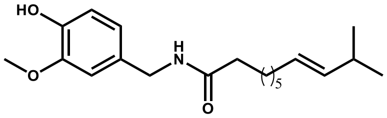
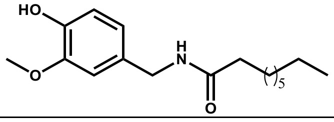
Chapter 1 Introduction

1.1 Capsaicinoids

Secondary metabolites produced by *Solanaceae* plants such as peppers, tomatoes and potatoes are natural defenses against animals, insects and fungi [1]. Due to their high pungency, capsaicinoids produced by peppers have found wide spread use in modern society for various reasons including the preparation of spicy foods [2], medicinal creams [3], and self-defense products (i.e., pepper sprays) [2–4]. The various pharmacologic responses to capsaicinoids (e.g., analgesic effects, anti-inflammatory activities, temporary blindness, and an intense burning sensation) are mainly attributed to reactions involving capsaicin, and dihydrocapsaicin (Table 1) [5–8].

Since the adoption of capsaicin-based products many adverse effects have been reported following exposure [9–11]. This has triggered the development of various methods to assess pungency. In 1912, Wilbur L. Scoville introduced the Scoville Heat Units (SHU) as the standard for measuring pungency in various pepper fruit types. The concept was developed along with the Scoville Organoleptic Test [12]. Scoville's method dissolved dried chilies in alcohol and diluted it with sugar water [12,13]. A five-person panel analyzes this solution and three of the five heat samplers must agree on one heat value, SHU. The subjectivity of the Scoville method has caused it to be replaced by high-pressure liquid chromatography (HPLC)[12,13]. This analytical method is currently preferred by the food industry because of its consistency compared to the Scoville Organoleptic Test. Though effective, this analytical technique, along with others, are either time consuming, costly, or subject to interference. For example, colorimetric/spectrophotometric methods have become popular, [14–16] but require extensive solvent extraction and subsequent removal of chlorophyll interference by column chromatography. Other reported analytical methods include complexation chromatography, [17] enzyme-linked immunosorbent assay, [18] gas chromatography (GC), [19] and mass spectrometry (MS) coupled to GC or HPLC [20–22]. As expected, these advanced methods provide a more accurate description of safe exposure levels of capsaicinoids, but their drawbacks include long analysis times and high solvent consumption. Therefore, simple and rapid analytical methods, which consume small amounts of sample and require minimum sample preparation, are still desirable.

Table 1. Chemical structure, relative amount and the corresponding level of pungency for capsaicinoids found in pepper extract

Capsaicinoid	**Chemical Structures**	**Relative Amount (%)**	**Pungency Level (SHU)***	**Molecular Weight (Da)**
Capsaicin		69	16.0×10^6	305.4
Dihydrocapsaicin		22	15.0×10^6	307.4
Nordihydrocapsaicin		7	9.10×10^6	293.4
Homodihydrocapsaicin		1	8.60×10^6	321.5
Homocapsaicin		1	8.60×10^6	319.4
Nonivamide			9.20×10^6	293.4

*SHU = Scoville heat unit = (mg capsaicinoid per gram of dry pepper) × 1.7×10[17]

1.2 Ambient Ionization

Ambient ionization [23–25] was invented by the Cooks group in 2004 through the introduction of desorption electrospray ionization (DESI) [26], shown in Figure 1a, which utilized charged micro-droplets for in-situ analyte extraction [27,28] and enabled MS analysis of unmodified samples in open air. Instead of probing the sample with electrosprayed charged droplets, other chemical/physical agents such as plasma [29–31]

(Figure 1b) and laser [32–34] (Figure 1c). The common feature among all ambient ionization methods is that they require no or minimal sample preparation providing almost instantaneous results. This capability permits field analysis with handheld mass spectrometers [35–39].

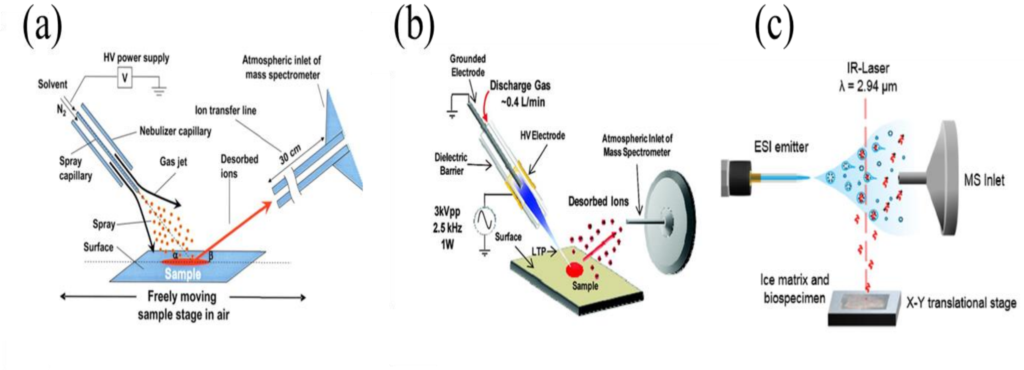


Figure 1. Schematic showing numerous ambient ionization sources (a) desorption electrospray ionization [36] (b) low-temperature plasma ionization [36] (c) infrared matrix-assisted laser desorption electrospray ionization [40]

High gas and solvent flow have limited the use of DESI as a source for miniature mass spectrometry [36]. This has led to the development of a new class of methods, like those shown in Figure 2, which rely on substrates such as paper, [41–44] blade [45], wood [46] or that generate ions from a sharp tip (naturally present or created). These substrate-based methods incorporate a sample preparation step in the ionization process [24]. Solid or liquid samples are deposited onto the surface of the substrate then wetted with solvent [47]. Once wetted, high voltage is applied to the substrate causing a high field at the sharp tip generating an electrospray [47].

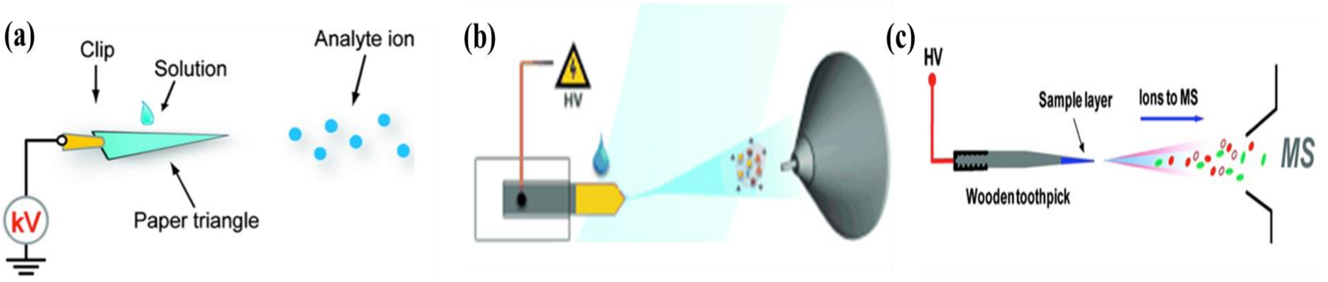


Figure 2. Schematic showing various substrate-based ambient ionization sources (a) paper [36] (b) blade spray [45] (c) wood [36] that can potentially be coupled with mass spectrometry

1.3 Thread Spray Ionization

The thread spray experiment presented in this thesis is similar to substrate-based the ambient ionization methods mentioned above in that a single thread pulled from a fabric is used as a substrate for analyte ionization. Some advantages exist for thread spray ionization when compared with other substrate-based ambient ionization methods: (i) it is capable of ionizing analytes present in solution or previously deposited onto a fabric, presenting a unique opportunity to directly analyze pepper spray residues on clothing for forensic applications; (ii) unlike metal substrates, the porous organic threads can provide selective absorption of components in a mixture and allow in-situ separation during analysis; (iii) various off-the-shelf thread types are available as substrate for analysis and will create a large degree of flexibility in experimental design; (iv) surface derivatization of thread surface is possible, which will enable the immobilization of various functional groups for enhanced analytical performance.

The aim of this research is to develop and establish the thread spray ionization method for direct mass spectrometric analysis of capsaicinoids in complex mixtures with no sample preparation. The developed method was utilized for the detection of (i) capsaicinoids in pepper spray for forensic application and (ii) capsaicinoids in intact pepper fruits. Sampling was achieved with a single thread; application of the direct current (DC) voltage to the wet thread allows the generation of charged liquid droplets containing the analytes, which are subsequently transferred to mass spectrometer for characterization.

Chapter 2 Methods

2.1 Thread Spray Ionization Apparatus

The developed thread spray ionization method (Figure 3) is simply made up of a thread (30 mm) inserted into a glass capillary (1.63 mm OD and 30 mm in length). The threads were pulled from the corresponding fabrics (100% cotton, 100% polyester, nylon and cotton:polyester (35/65) blend) on which the sample (i.e., pepper spray) had previously been deposited. The small OD (ranged 225.315 – 302.220 µm) of the thread and good electrical conductivity when wetted enable the tip to be conveniently fitted in front of a mass spectrometer with no need for modification/sharpening.

This apparatus allows for two possible experimental setups: (i) a solution (20 µL) of the analyte of interest is placed in a glass capillary and electrically charged via contact with the thread to which a high DC voltage has been applied. This experiment is similar to nano-electrospray ionization except that the tip of the glass capillary need not be sharp and requires higher voltage (3-5 kV). Application of the DC voltage to the wet thread allows the generation of charged liquid droplets containing the analytes (Figure 4), which are subsequently transferred to mass spectrometer for characterization. This procedure was used for the optimization of the thread spray ionization experimental setup. (ii) In a second set of experiments, the sample (pepper fruit or pepper spray aerosol) was deposited on the fabric and allowed to dry. A thread was pulled, cut and then inserted into the glass capillary. Residues coated on the pulled thread was eluted with 20 µL acetonitrile followed by in-situ thread spray ionization.

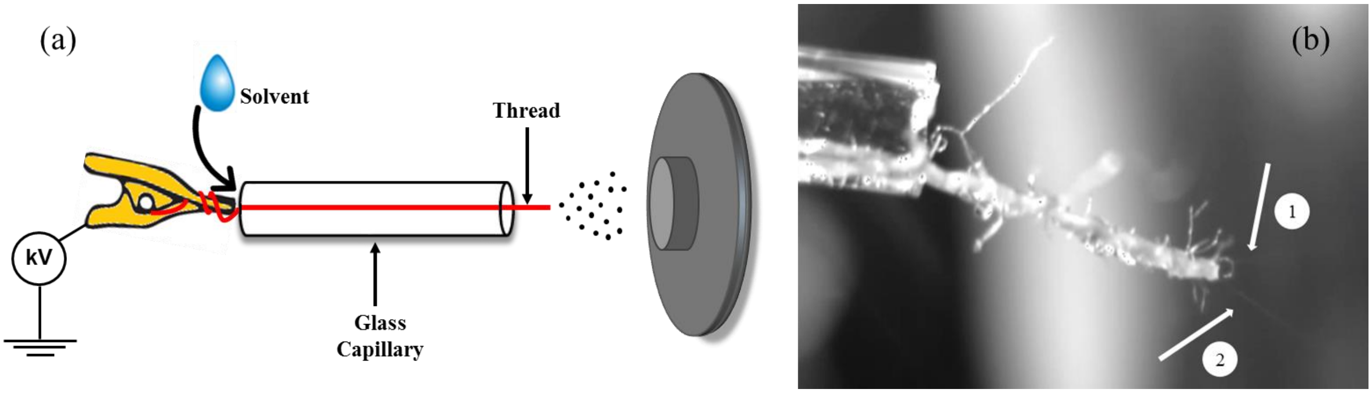


Figure 3**.** (a) Schematic diagram of the experimental setup of thread spray ionization (b) Thread spray ionization setup and formation of Taylor cones (1) and (2)

2.2 The Mass Spectrometry

Mass spectra were acquired on a Thermo Fisher Scientific Velos Pro LTQ linear ion trap mass spectrometer [48] (Figure 4) and high resolution mass spectra were acquired on a Thermo Fisher Scientific LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap mass spectrometer [49] (Figure 5) (San Jose, CA, U.S.A.). The tip of the thread was held and positioned parallel to the MS inlet via a copper alligator clip, which was connected to an external high-voltage supply (0 – 6 kV). The thread spray ionization method generated ions without gas assistance, and thus, a close interface distance (0.5–5 mm) between the tip and the MS inlet was used to optimize signal intensity. MS parameters used were as follows: 200 °C capillary temperature, 3 microscans, and 60% S-lens voltage. Thermo Fisher Scientific Xcalibur 2.2 SP1 software was applied for MS data collecting and processing. Tandem MS with collision-induced dissociation (CID) was utilized for analyte identification. An isolation window of 1.5 The (*m/z* units) and a normalized collision energy of 30%–35% (manufacturer's unit) were selected for the CID experiment.

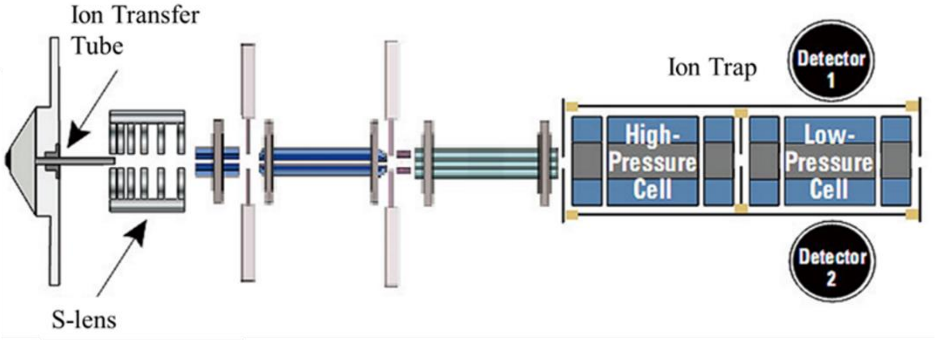


Figure 4. Scheme of the Thermo Fisher Scientific Velos Pro LTQ mass spectrometer used in these experiments [48]

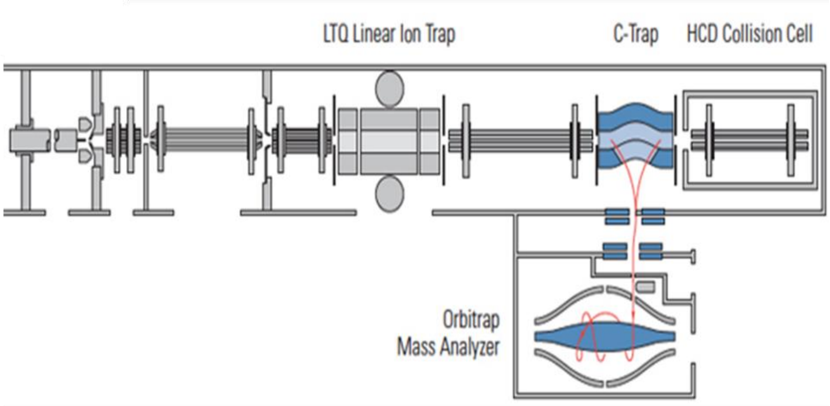


Figure 5. Scheme of the Thermo Fisher Scientific Velos Pro LTQ mass spectrometer used in high resolution experiments [49]

2.3 3-D Laser Scanning Confocal Microscopy

3-D surface topographic measurements of thread were analyzed and captured using the Keyence 3-D Laser Scanning Microscope VK-X200 [50] (Itasca, IL, U. S. A) (Figure 6). Wetted thread was placed on a microscope slide onto the stage then adjusted and focused with 20x objective lens, where thread diameter and sub-fibers were measured. At 20x power objective, optical laser images of the thread surfaces were taken. 2-D imaging occurs by using high-frequency XY laser as a light source with lateral resolution of 408 nm. Thread sample was then observed using 50x objective lens, where thread topographic analysis was measured. 3-D topographic imaging and surface analysis were achieved using objective lens moving in the z direction.

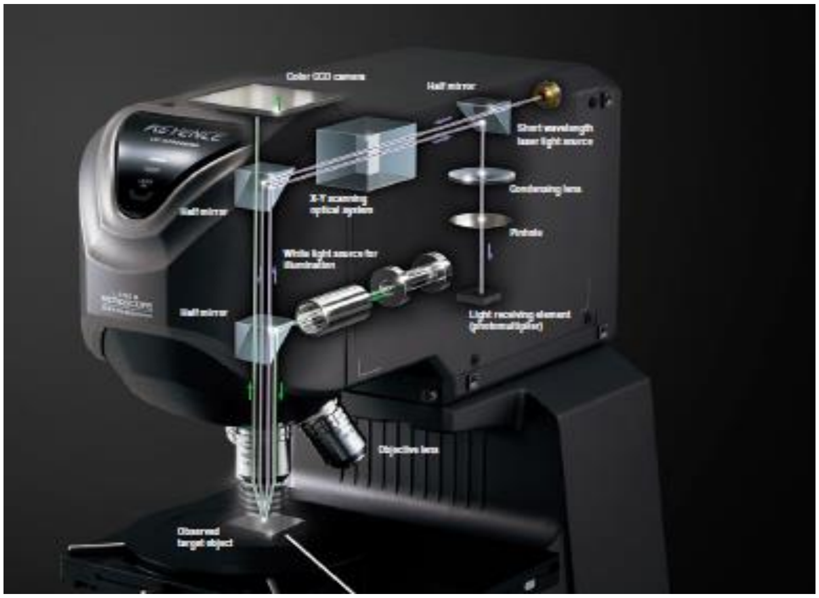


Figure 6. Scheme of the Keyence 3-D Laser Scanning Microscope VK-X200 used for thread surface roughness analysis [50]

2.4 Reagents

Capsaicin, acetonitrile (99.9%, HPLC grade) and methanol (99.9%, HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Methylene Chloride (99.9%, HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ). Nylon, 100 % Polyester, Cotton:Polyester (35:65), and 100% Cotton were purchased from a local store (JoAnn Fabrics, Columbus, OH). Kimble 51 expansion borosilicate glass melting point capillaries (O.D. 1.5 mm) were purchased from Kimble Chase (Rockwood, TN).

Chapter 3 Results and Discussion

3.1 Optimization

This thesis introduces a novel thread spray ionization mass spectrometry (MS) method. It includes utility, development and characterization using the analysis of capsaicinoids in complex mixtures (e.g., pepper fruits and pepper spray) with no sample preparation. The method was first optimized by investigating the following: ion signal, solvent effect, voltage effect, thread composition, and surface roughness. These parameters were investigated using pure capsaicin (1 ppm) prepared in acetonitrile. In particular, the method was characterized using the pure capsaicin and four different threads (100% cotton, 100% polyester, nylon and cotton/polyester (35/65%) blend). The optimized parameters were applied for the analysis of several different peppers fruits (bell pepper, serrano pepper, red chili pepper, ghost pepper, habanero, and jalapeño) and pepper spray mixture from which all five capsaicinoids were detected. The method is quantitative, fast, and robust capable of detecting dried capsaicinoids after several weeks of storage.

3.1.1 Ion Signal

Ion chromatogram produced by mass spectrometers are composed of a large set of consecutively acquired mass spectra where each component can be identified from the mass spectrum. The total ion chromatogram (TIC) measures both the mass spectral output as a function of time and the overall intensity of ion production[51–53]. Figure 7 shows total ion chromatogram persisted for more than 5 minutes after 5 kV DC voltage was applied to the wet thread.

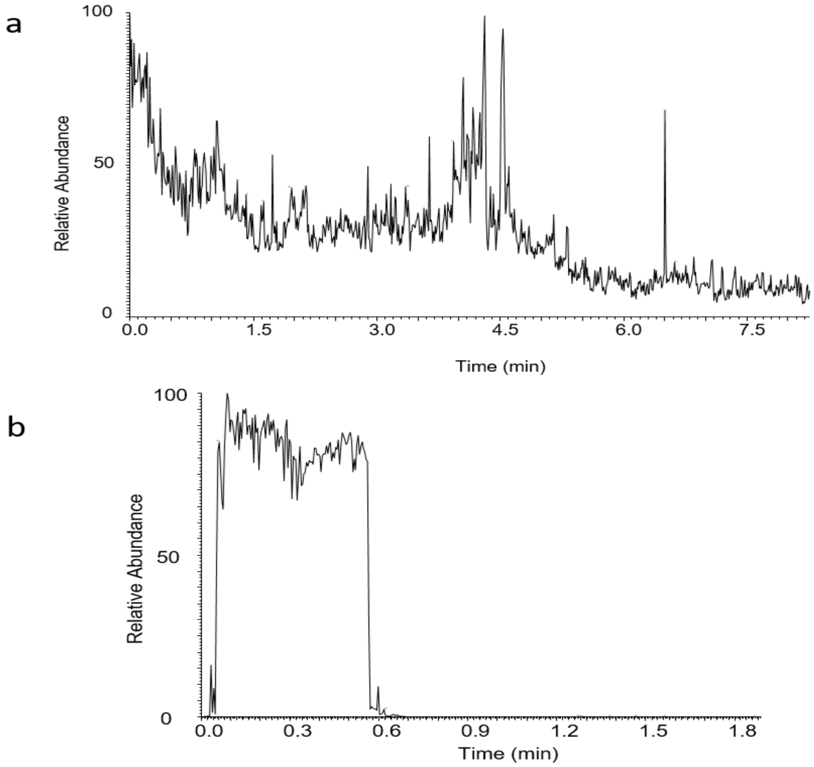


Figure 7. Total ion chromatogram observed after 5 kV DC voltage applied to (a) wet thread and (b) wet fabric triangle. (a) Ion signal of 20 µL acetonitrile solvent remained for more than 7 minutes. (b) Ion signal of 20 µL acetonitrile solvent on fabric triangles remained for 0.6 min.

The 20 µL acetonitrile solvent lasted for more than 4 min after which ion signal was continuously observed from the wet thread for another 3 min although the ion current drastically reduced after the 4 min. This signal lifetime is >5x longer than analysis performed from a fabric cut into a triangle, which sustained ion signal for only 0.6 min. This performance of the fabric spray is comparable to what has been reported for paper spray [42,54]. The longer analysis time for the thread spray experiment is attributed to limited solvent evaporation in the glass capillary. The large surface area for the fabric facilitates solvent spreading leading to rapid evaporation. Like paper spray, contribution from atmospheric pressure chemical ionization can be expected in thread spray ionization especially after the solvent is consumed[55].

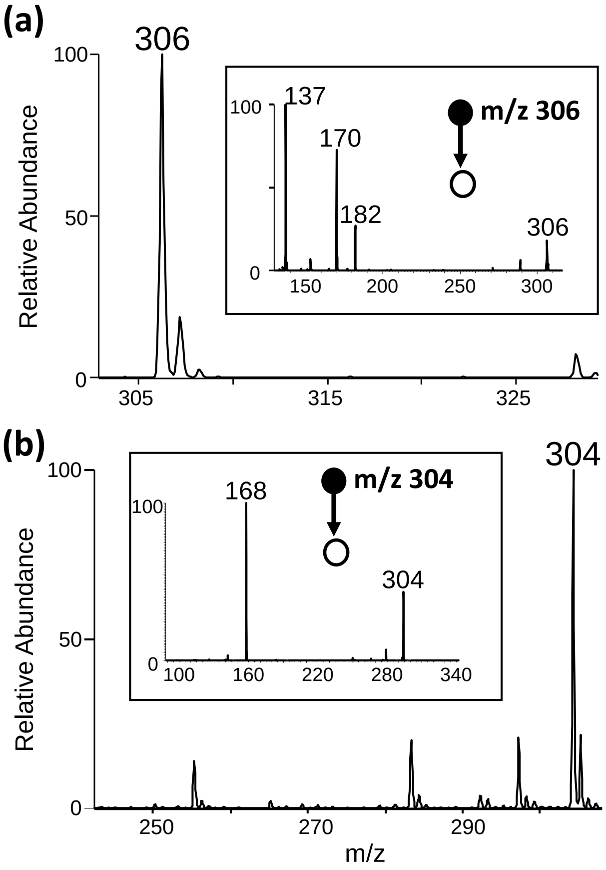
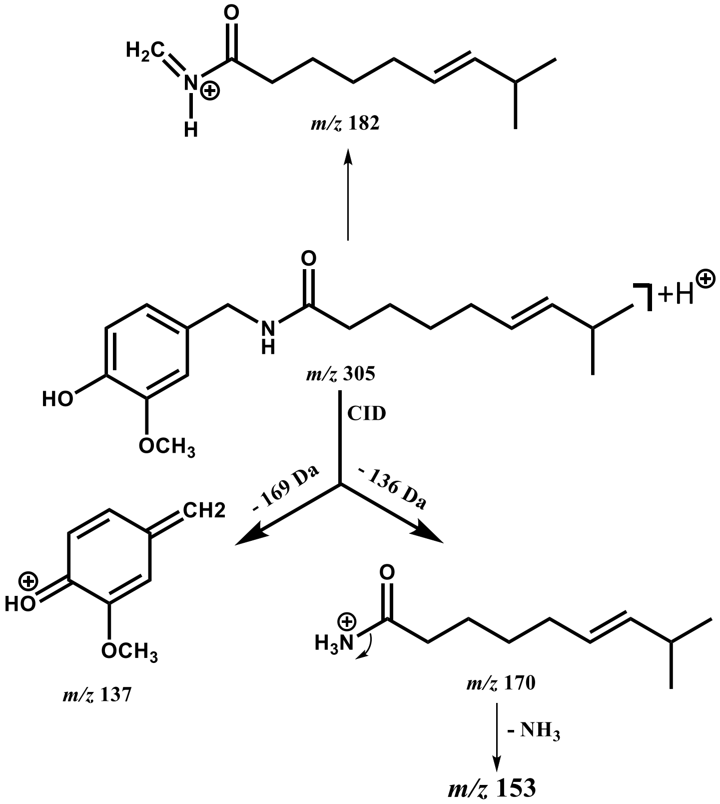


Figure 8**.** Thread spray mass spectra recorded for pure capsaicin (*MW* 305) prepared in acetonitrile solution (20 µL) in **(a)** positive and **(b)** negative ion modes using cotton:polyester (35/65) thread charged at ±5 kV. Inserts show CID MS/MS spectra for both protonated (*m/z* 306) and deprotonated (*m/z* 304) capsaicin

The protonated $[M+H]^+$ capsaicin ions were clearly observed at *m/z* 306 when using positive voltage (+5 kV) (Figure 8a). The weak acidic nature of the phenolic moiety allows MS detection in the negative mode through the generation of $[M–H]^-$ ions (Figure 8b). The structure of capsaicin ions was confirmed through CID MS experiments. The negatives ions fragmented predominantly through the elimination of a neutral phenolic head group (*MW* 136) to give ion at *m/z* 168 (Figure 8b insert). The fragmentation pattern for the positive protonated ions under CID is as shown in Scheme 1, and the corresponding spectrum is provided in the insert of Figure 8a where the major cleavages occurred at the phenyl and benzyl positions; in this case, the resonance stabilized phenolic cation (*m/z* 137) was the predominant fragment ion.



Scheme 1. CID fragmentation pattern of protonated capsaicin in positive ion mode MS/MS showing the pathway to major fragment ions m/z 137, 170, 182 and 153

3.1.2 Voltage and Thread Type

Using positive ion mode analysis, the effect of thread type and spray voltage on the formation of protonated ions was investigated. Figure 9 shows the optimum voltage for nylon and 100 % polyester threads occurred at 4 kV while 100% cotton and cotton:polyester (35/65) threads required 5 kV for optimum performance.

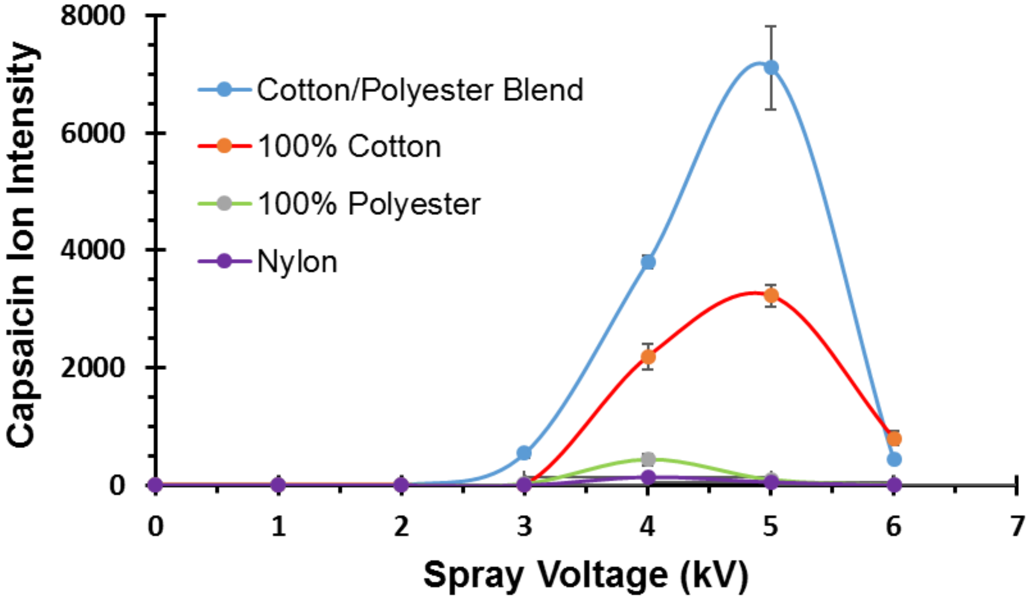


Figure 9. A plot showing the effect of tread type and spray voltage of capsaicin ion $[M+H]^+$ intensity when using acetonitrile spray solvent.

Although ion formation occurs from tiny sub-fibers at the tip of the thread (Figure 3b), where electric field is highest, the higher spray voltage requirement is not surprising given that analyte flow from the bulk sample solution occurs through the thread, which is about 220 – 300 µm in size (Table 2). As shown in Figure 10, spray voltage is effected by thread type with cotton:polyester 35/65 having the highest ion intensity and 100 % polyester and nylon having the lowest.

Table 2. Thread diameters for cotton:polyester 35/65, 100% cotton, polyester, and nylon measured using 3D laser scanning confocal microscope.

Thread	**Diameter (µm)**
Cotton:Polyester (35/65)	302.220
100% Cotton	296.313
100 % Polyester	225.314
Nylon	N/A

N/A: could not be measured using scanning confocal microscope due to its soft nature and inability to remain intact.

Factors such as geometrical effects that affect ionization efficiency in paper spray mass spectrometry ionizations [41–43,56] are not relevant in thread spray ionization. For example, Table 2 provides the diameters of the different threads (except for nylon with inconclusive measurement). The diameter measurements obtained with the 3D laser scanning confocal microscope for cotton:polyester 35/65, 100 % cotton, 100 % polyester, and nylon were found to be very similar meaning the differences in ionization efficiencies observed in Figure 9 are not influenced by the diameter of the threads. Furthermore, close examination of the data from 3D laser scanning confocal microscopy revealed that the diameter of the sub-threads (Figure 10, red dots; average size is 16 µm per sub-fiber) of the various thread types are also very similar. Therefore, it can be argued that the overall diameters of different thread types do not play an important role in the overall ionization mechanism of thread spray mass spectrometry. In fact, during the ionization process, small fibers can be seen excreting small Taylor cones (Figure 3b) on the thread sub-fibers just like that observed for paper spray ionization[41–43].

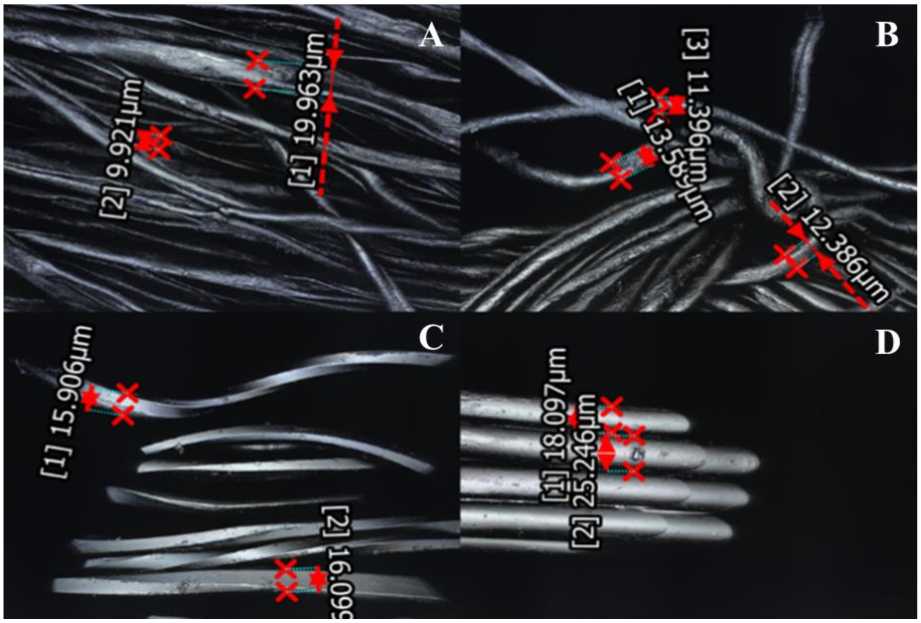


Figure 10. 50x 3D laser images of thread surfaces (a) cotton:polyester 35/65, (b) 100 % cotton, (c) polyester and (d) nylon measured using a 3D laser scanning confocal microscope

3.1.3 Ion Efficiency

Ionization efficiency is greatly impacted by solvent choice for ambient ionization methods [57]. To enhance ionization of analyte, one can optimize spray solvent to match the solubility of the analyte of interest. Other ways of improving ionization efficiency is through chemical modification of analyte [28,57,58]. Solvent choice is essential for efficient ionization but thread spray ionization is heavily affected by the substrate composition as seen from the various thread types in which markedly different ion intensities were observed. This reason marks the difference in performance between thread types, but the conductivity of thread (determined by chemical composition) and mobility of analyte through the thread play a role.

Therefore, spray solvent and thread compositions are important for optimum sampling in thread spray mass spectrometry. A good solvent for thread spray ionization should have the following properties: (i) be suitable for electrospray ionization [59], (ii) be able to wet the thread to increase its charge conduction [57], and (iii) have high solubilizing power for analyte and act as a "mobile" phase by reducing analyte-thread interactions [60]. Due to the poor solubility of capsaicin in water, organic solvents such as acetonitrile, methanol, and dichloromethane were used. The aprotic polar acetonitrile [61] spray solvent was superior for capsaicin analysis from cotton:polyester (35/65) thread (Figure 9).
Although resistivity measurements did not indicate major changes in conductivity between thread types (Figure 11), it is believed that the blending of hydrophilic cotton with the low surface energy hydrophobic polyester threads produced a material that effectively transports capsaicin when wetted by a polar solvent. The cotton/polyester blend can be expected to be less porous than the 100% cotton material and so inclusion is limited allowing more analyte to be delivered to the mass spectrometer. The poor performance for nylon and 100% polyester in ionizing capsaicin is attributable to their low surface energies that limit wetting by the polar acetonitrile solvent; ionization capacity for both threads was increased more than 10x when utilizing dichloromethane as spray solvent as seen in Figure

12 although the total ion intensities were still less than that recorded from 100% cotton and cotton:polyester (35/65).

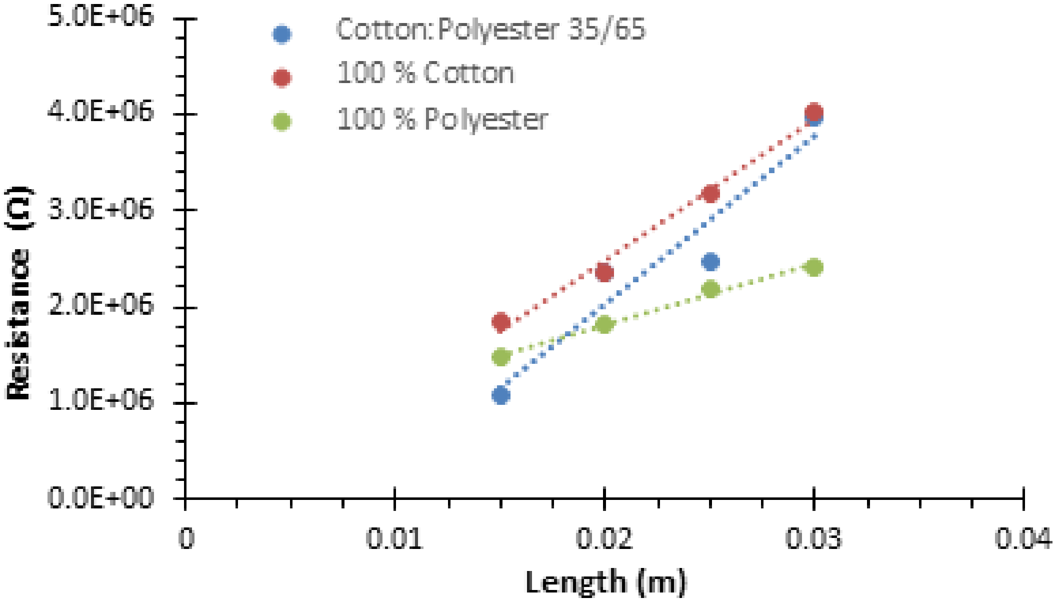


Figure 11. Resistance as a function of length of cotton:polyester 35/65, 100% cotton, 100% polyester

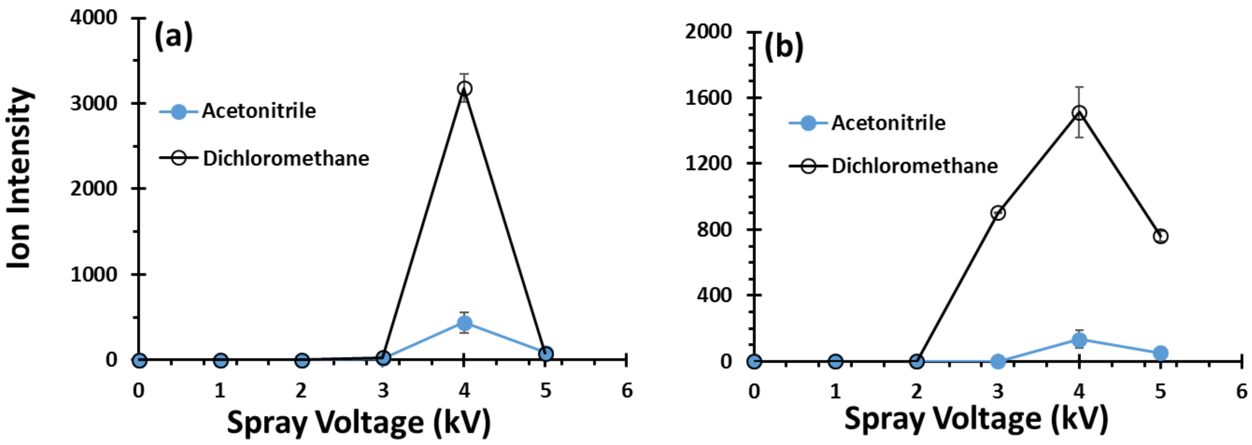


Figure 12. (a) The effect of polyester thread and spray voltage of capsaicin ion $[M+H]^+$ intensity when using acetonitrile and dichloromethane spray solvent. (b) The effect of nylon thread and spray voltage of capsaicin ion $[M+H]^+$ intensity when using acetonitrile

3.1.4 Surface Roughness

Solid surfaces are complex depending on the nature of the solid, surface preparation, and surface interactions. These surfaces contain irregularities in surface properties such as height, surface smoothness, and surface textures that can all be attributed to the surface roughness of a given solid surface [62]. These surface parameters can contribute to the interaction of the different threads types with capsaicin solution and ionization efficiency.

In these regards, the characterization of the various thread types was achieved through surface roughness studies. Thread surfaces are highly irregular; these irregularities can lead to differences in the solid-liquid interaction between analyte and fiber surface roughness [63]. As shown in 3D laser microscopy images in Figure 13, the surfaces of polyester and nylon threads are smooth with uniform and loose sub-fibers while the cotton:polyester and 100% cotton thread are more rigid in comparison. These observations are confirmed by the surface roughness measurements summarized in Table 3, also obtained by 3D laser scanning confocal microscope.

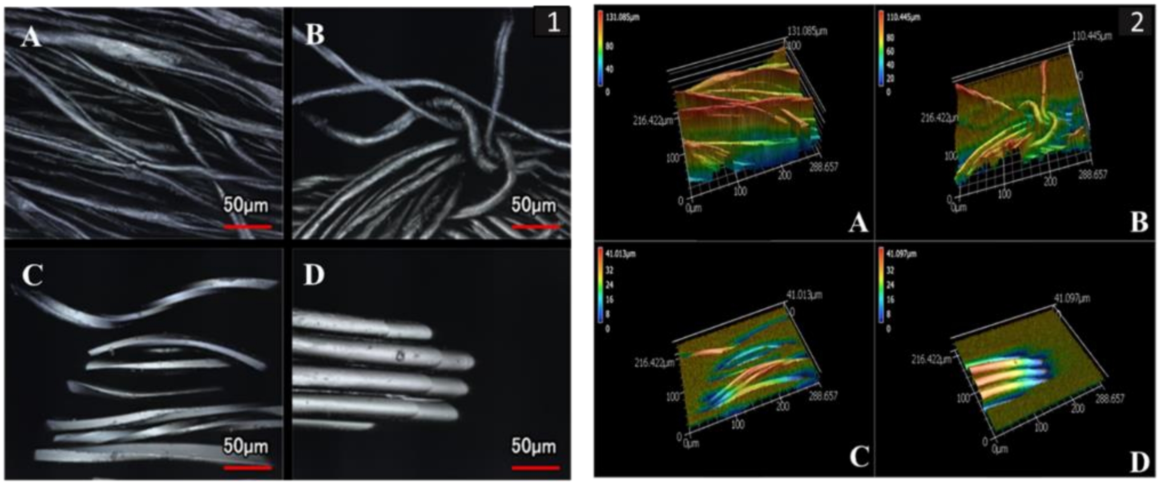


Figure 13. (1) 50x optical laser images and (2) 3D surface height image of (a) cotton:polyester 35/65 (b) 100 % cotton (c) 100% polyester and (d) nylon thread using a 3D laser scanning confocal microscope

Surface Roughness	**Sa (µm)**	**Sz (µm)**	**Str**
Cotton:Polyester 35/65	26.2	131.0	0.3
100 % Cotton	13.4	110.2	0
Polyester	9.0	40.8	0.4
Nylon	7.3	40.9	0

Table 3. Quantitative thread surface roughness features for cotton:polyester 35/65, 100% cotton, polyester, and nylon

Both S_a, which is the arithmetic mean height of the surface, and S_z, which is the maximum height of the surface, show cotton:polyester 35/65 having the largest surface height compared to 100 % cotton, 100 % polyester and nylon. Str, measures the systematic creases or surface texture on a given surface, with a value ranging from 0-1. Smaller values indicated more clear creases. It is expected for nylon to have a Str of zero due to its inability to bond during analysis. 100 % cotton clear creases can be seen in the optical image acquired in insert of Figure 13, 2b, where the uniformity of cotton is seen. Polyester, has an intermediate texture compared to 100 % cotton and nylon leading to a larger value of ~0.4. Since cotton:polyester 35/65 is made up of some cotton, less textured, and polyester, intermediate textured, its value lies between cotton and polyester. Str for all four are still relatively low and no real differentiation can be made using this parameter. The increased surface roughness seen in threads such as cotton:polyester and 100% cotton leads to the strong wettability [64] of the thread fibers. The surface wettability is increased with a large surface roughness because of the increased surface energy of the thread compared to that of the liquid surface tension. This large surface energy will allow for wetting of the thread leading to increased extraction. In contrast, nylon and polyester threads have minimum surface roughness, which increases their interaction with capsaicin leading less extraction and lower ionization efficiency. Therefore, the increase in surface irregularities can have an effect of enhancing surface interactions and ion production [62,63].

3.2 Thread Spray Mass Spectrometry Applications

The thread spray experimental setup was utilized for direct analysis for capsaicinoids in complex pepper spray residues and pepper fruit multiplex analysis. This novel ambient ionization method allows direct mass spectrometric detection of capsaicinoids from a single thread with no need for sample pretreatment. Cotton:polyester (35/65) threads were utilized for these experiments containing undiluted pepper spray using acetonitrile spray solvent and 5 kV of DC voltage. Pepper spray was also stored on thread for a period of time in different environments to assess thread spray robustness.

Using the same thread spray MS method, the detection of capsaicinoid content in fresh pepper fruits was straightforward and required no or little fruit destruction. Here, the capsaicinoids were sampled using a thread suspended on a needle. By pushing the needle through the pepper fruit, capsaicinoid residues were collected on the thread, which was subsequently cut into several pieces and sampled in front of the mass spectrometer after placement in the glass capillary and applying a DC voltage to the wet thread. Thread spray method could potentially have a significant impact on the food industry and forensic field by becoming a sensitive, robust, and rapid onsite analytical method.

3.2.1 Direct analysis of pepper spray

Pepper sprays are complex mixtures of capsaicinoids that are utilized by both law enforcement personnel and the general public for riot control and self-defense purposes[65]. The presence/absence of pepper spray on an evidentiary garment may help establish the realities of the incident. Colored dyes are typically added to pepper spray mixtures to assist in locating the position of capsaicinoids but traditional analytical methods are time consuming as they require separate extraction steps to enable effective characterization of the capsaicinoids. The laborious process of traditional techniques usually leads to a backlog of evidence and delay in the judicial process. Figure 14 shows mass spectrum recorded from cotton:polyester (35/65) thread containing undiluted pepper spray using acetonitrile spray solvent and 5 kV of DC voltage. Five capsaicinoids were detected in the full mass spectrum, including the minor components homodihydrocapsaicin and homocapsaicin at *m/z* 322 and 320, respectively. Confirmation of the structure of the detected ions were accomplished through tandem mass spectrometry. All ions fragmented by breaking the C-N bond at the benzylic position yielding protonated species of the diagnostic phenolic head group at *m/z* 137 accompanied by peaks corresponding to the linear alkyl groups. This common fragmentation pattern made it easy to confirm the presence of the low abundant capsaicinoids from all thread types tested. The ability to achieve multiplexed detection of five capsaicinoids in a single experiment add credence to the proposed method by reducing false positive identification.

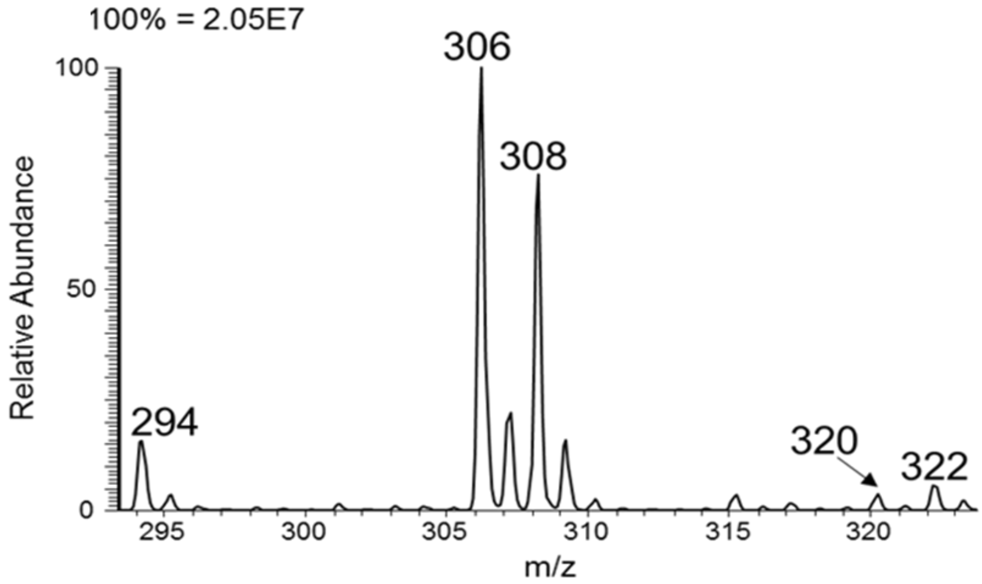


Figure 14. Full MS of pepper spray using 65:35 cotton: polyester thread, acetonitrile and 5 kV in positive mode. MS-MS of pepper spray using 65:35 cotton: polyester, acetonitrile and 5 kV in the positive mode

The above results are significant because in-situ capsaicinoid analysis is facilitated by reducing sample preparation. In addition, the current thread spray MS detection method has potential to reduce backlogs of evidence through onsite analysis (when coupled to portable mass spectrometer) or by easing storage of evidentiary garments in the form of a threads. To test this possibility, threads containing 10% pepper spray residues were stored in air, at room temperature in a closed drawer for 10 weeks. After each week, the stored threads were analyzed using the thread spray MS method. Although reduced signal (>10x) was detected for the stored capsaicinoids when compared with fresh pepper spray aerosol (Figure 15) all five capsaicinoids including the minor species like homocapsaicin were easily detected in high abundances after the ten weeks storage. This demonstrates the likelihood of storing pepper spray-contaminated threads for even longer periods if no prior dilutions are made. Material destruction is also minimized when detecting the presence of capsaicinoids from thread.

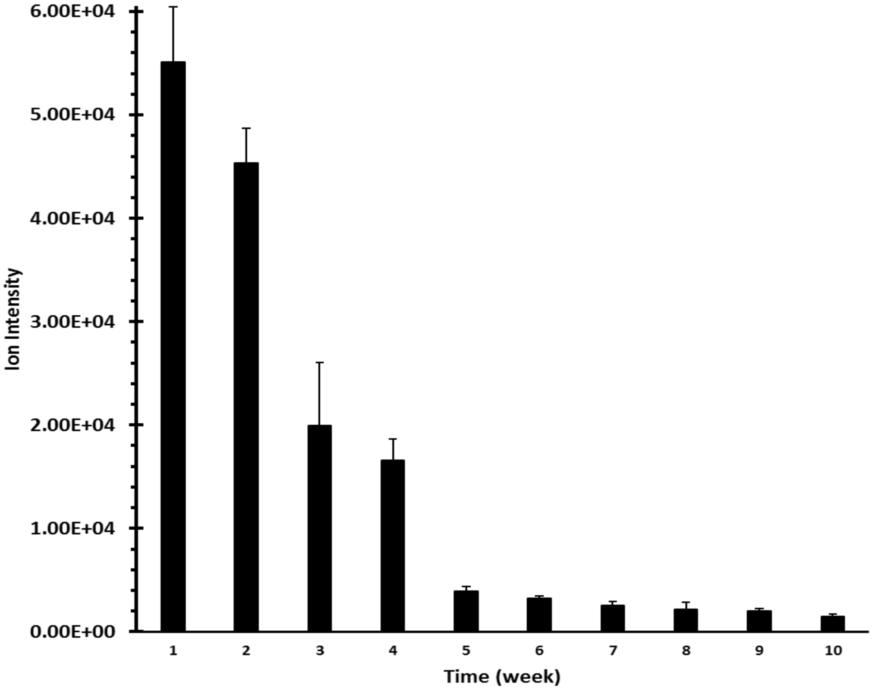


Figure 15. Robustness analysis of stored pepper spray solution on 65:35 cotton: polyester thread over 10 weeks

Over the course of time, the analysis of capsaicin on stored cotton:polyester (35/65) threads decreases (Figure 15). To determine if the decrease in signal is due to storage conditions, and not an artifact of the thread spray method, pepper spray residues were stored on thread in different environmental conditions (Figure 16): at room temperature in a closed drawer, 4 ° C in a closed drawer, and under vacuum in a desiccator at room temperature, for a total of four weeks. Each week threads were analyzed and compared to fresh pepper spray in acetonitrile solution and pure pepper solution, without dilution.

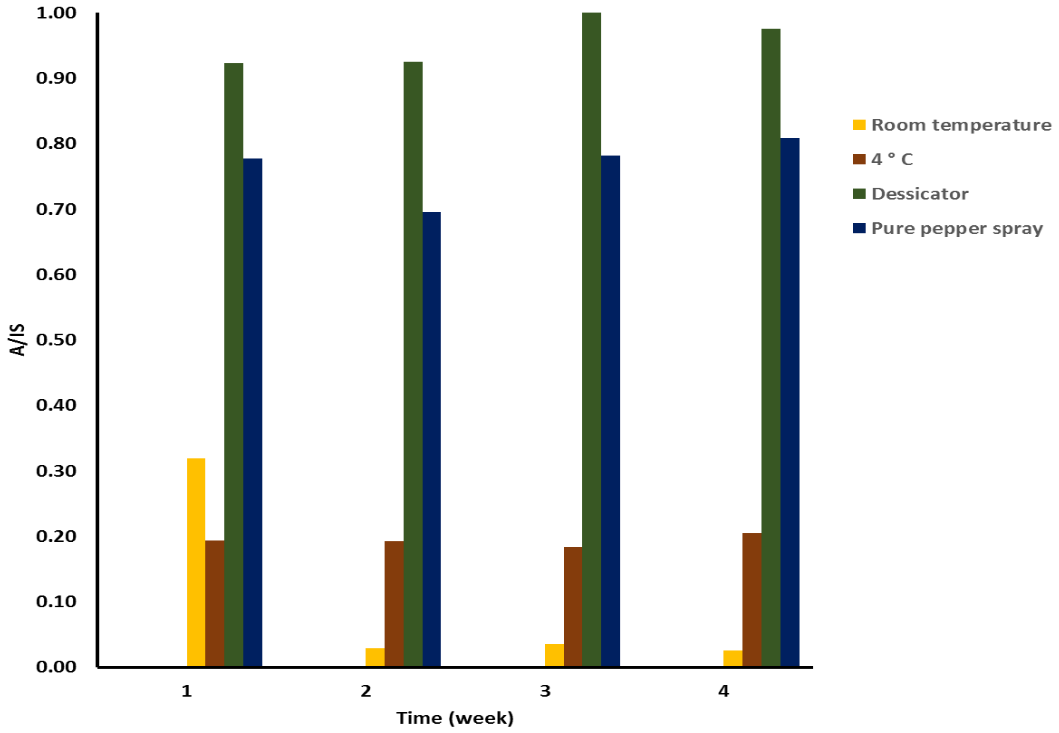


Figure 16. Robustness analysis of stored pepper spray solution on 65:35 cotton: polyester thread in different environments for one month; A/IS, fresh pepper spray was used as an internal standard

Figure 16 shows that the residue of acetonitrile/pepper spray mixture on threads kept at room temperature were very unstable following week one while pure pepper spray residue (undiluted) (blue bars, Figure 16) threads kept under the same environmental conditions were consistently stable over the course of the four weeks. This is due to the high concentration of capsaicin in pure pepper spray compared to the pepper spray in acetonitrile solution. The high concentration of capsaicin in pure pepper spray form more stable complexes on thread. The pepper spray mixture diluted with acetonitrile solution gradually becomes unstable due to oxidation in air, and possible pepper spray aggregation in acetonitrile [66]. However, capsaicinoid degradation was drastically reduced by storing the same acetonitrile diluted pepper spray mixture under air-tight conditions, inside a desiccator (green bars, Figure 16) suggestion oxidation by air is the most prominent mechanism for capsaicinoid loss. Reducing the storage temperature also reduced pepper spray decomposition (brown bars, Figure 16), although overall ion intensity was low.

3.2.2 Pepper Fruit Analysis

Thread spray MS mass spectrum recorded for serrano pepper fruit is shown in Figure 17 in which all the five detectable capsaicinoids were observed in high abundance. Interestingly, the recorded relative ion intensities are in accordance with reported natural abundances for capsaicinoids in µg/g [67]. Figures 17b and 17c show tandem MS analysis of protonated species of dihydrocapsaicin (*m/z* 308) and homocapsaicin (*m/z* 320), respectively. Here too, both analytes followed the fragmentation pattern described in Scheme 1, with the protonated phenolic head group being the most abundant ion. For dihydrocapsaicin, however, protonation of the tail fragment (*m/z* 184) produced by cleaving the phenyl (C-C) position was more abundant than the corresponding ion (*m/z* 172) formed by breaking the benzylic (C-N) bond.

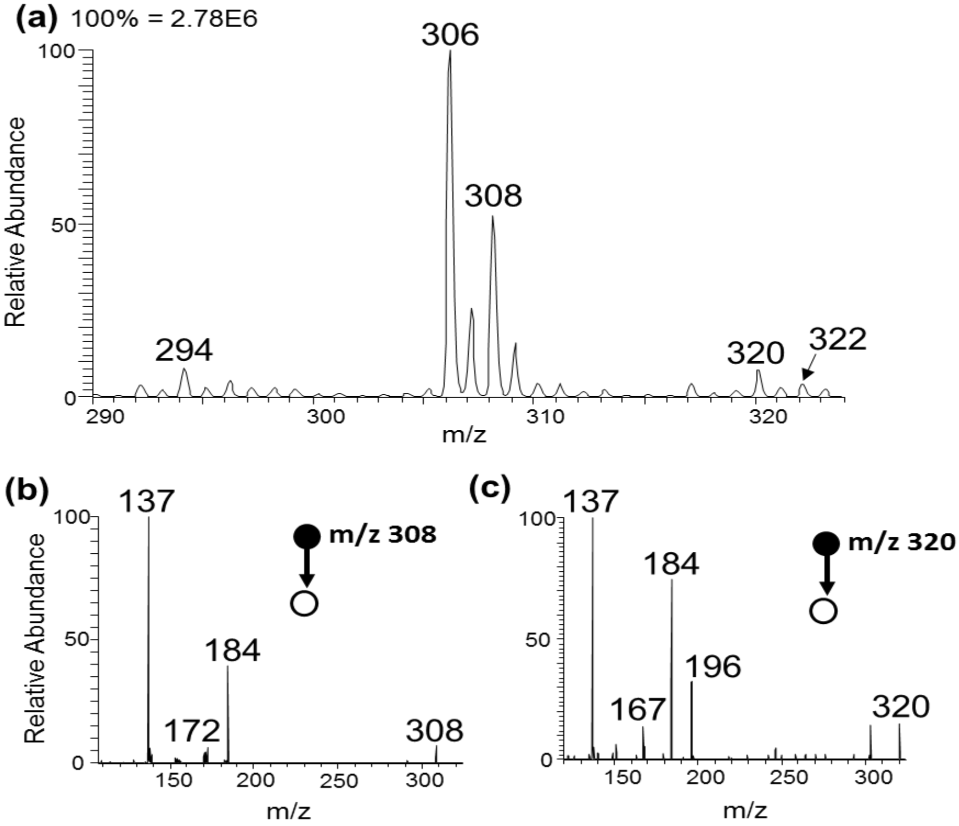


Figure 17. (a) Thread spray mass spectra showing various capsaicinoids present in green chili, serrano pepper. Tandem MS analysis of selected capsaicinoids including (b) dihydrocapsaicin (*m/z* 308) and (c) homocapsaicin (*m/z* 320).

Aside from the serrano pepper, the capsaicinoid content of 5 other pepper fruits (bell pepper, red chili pepper, ghost pepper, habanero pepper, and jalapeño) were analyzed in similar manner without breaking the fruits. The corresponding thread spray MS mass spectra are provided in Figure 18 in which all five detectable capsaicinoids were observed in the full MS and in the correct relative abundance as predicted in the literature. The bell pepper was also analyzed as a control because it is the only member of the *Capsicum* genus that does not produce capsaicinoids [68]. As such, no capsaicinoids were detected during thread spray MS. This new ambient MS technique show significant advantage over hyphenated methods (GC-MS and HPLC-MS) currently used in the food industry by offering simplicity in instruments and rapid sample analysis[69]. When compared with other substrate-based ambient ionization methods, the current experimental procedure provides a unique opportunity to non-invasively sampling the interior of fruits.

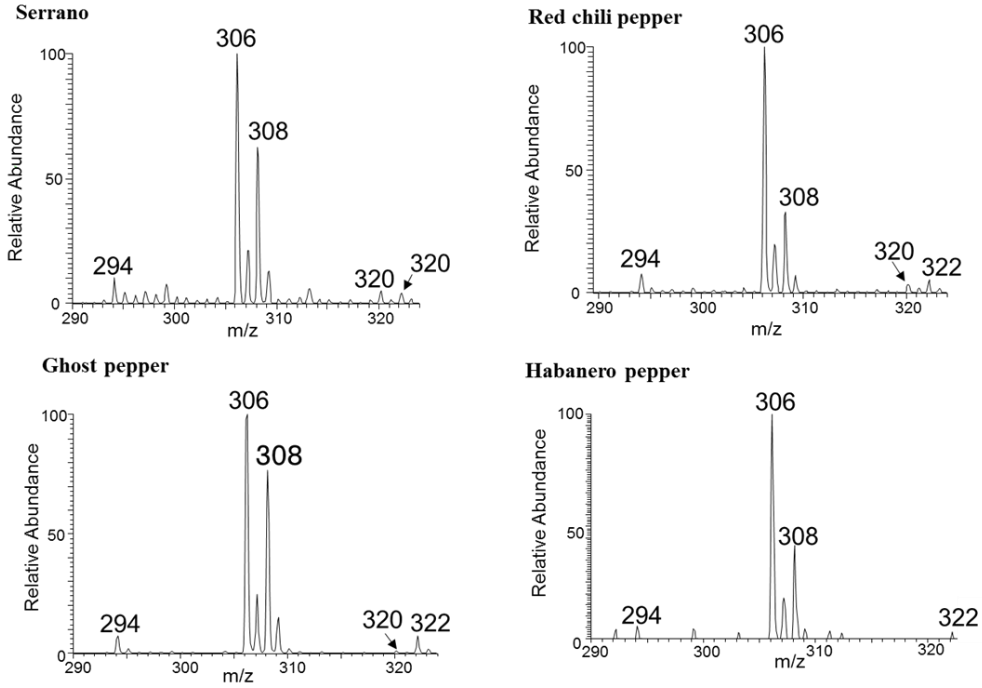


Figure 18. (a) Thread spray mass spectra showing multiplex analysis of various peppers (a) hot chili pepper (b) red pepper (c) jalapeño pepper. Acetonitrile spray solution (20 µL) was used and the cotton:polyester (35/65) thread was charged at 5 kV

Using acetonitrile spray solvent and cotton:polyester (35/65) thread charged at +5 kV good linearity ($R^2 = 0.992$) and acceptable precision (RSD <10%) were recorded for capsaicin standards (Figure 19). These results indicate that both qualitative and quantitative analysis of capsaicin can be achieved using thread spray MS. Limit of detection for capsaicin on thread spray is 1.48 µg/mL. Capsaicin and dihydrocapsaicin content in different pepper fruit genotypes range from 1.56 µg/mL to 5178.42 µg/mL[69]. This means that the sensitivity of thread spray will allow the detection of low concentration capsaicin and dihydrocapsaicin content in pepper fruits.

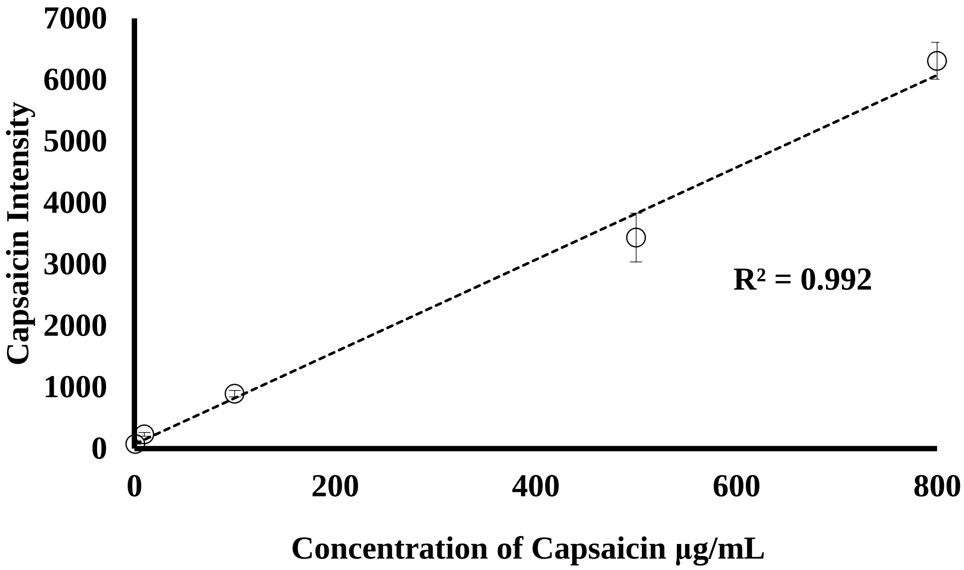


Figure 19. Calibration curve showing linear relationship between solution phase capsaicin concentration and gas-phase fragment ion (*m/z* 137) intensity. Both plots were obtained using cotton:polyester (35/65) thread charged at 5 kV

It is known that capsaicin and dihydrocapsaicin content are the most abundant in pepper fruits, and the main pungency factor is the level of capsaicin [69]. Utilizing the content of capsaicin alone can yielded accurate results for pepper fruits pungency. Pepper fruit pungency is effected by its genotype and the environment in which it is developed. These conditions can affect a pepper's overall capsaicinoid content and ultimately, it's Scoville Heat Unit. Having a miniaturized analytical method can help the development of pepper fruits growth by allowing for rapid analysis of capsaicin and dihydrocapsaicin levels in pepper fruits. As previously mentioned, traditional analytical methods cannot perform rapid analysis nor can they be miniaturized. Using the same method used for the multiplex detection of various pepper fruits, we can accurately determine the various capsaicinoid levels in pepper fruit and products with thread spray mass spectrometry (Table 4). Table 4 shows the level of capsaicinoids' ion intensities in five pepper products. Individual capsaicinoid levels in both the habañero and jalapeño pepper could not be correlated to the Scoville Heat Units (Table 4) using thread spray mass spectrometry. Fluctuating pepper capsaicinoid levels that occur naturally in peppers, may cause irregular measurements in pepper fruits.

Table 4. Capsaicinoids' ion intensities and total capsaicinoid ion intensities of various pepper fruits and pepper products

Pepper Type	C	DHC	NDHC	HDHC	HC	Total
U.S Grade Pepper Spray	9610000	170000	112000	23100	57600	10902600
Bhut Jolokia	322000	23800	23800	23100	32000	635900
Habañero	175000	12200	80500	926	2050	39286
Jalapeño Pepper	6360	22800	8850	4030	175	42215
Sweet Bell	0	0	0	0	0	0

Abbreviations of capsaicinoids: Capsaicin (C), Dihydrocapsaicin (DHC), Nordihydrocapsaicn (NDHC), Homodihydrocapsaicin (HDHC), Homocapsaicin (HC)

The total capsaicinoids' ion intensities in Table 4 were correlated with Scoville Heat Units for various pepper fruits and pepper products (Figure 20). By using the thread spray mass spectrometry method, all capsaicinoids residues can be analyzed and pepper fruit pungency can rapidly be determined. This method does not require extensive sample treatment like other analytical methods such as HPLC-MS or GC-MS[20–22]. It also can easily be coupled to a portable mass spectrometer meaning it is not limited to a laboratory setting.

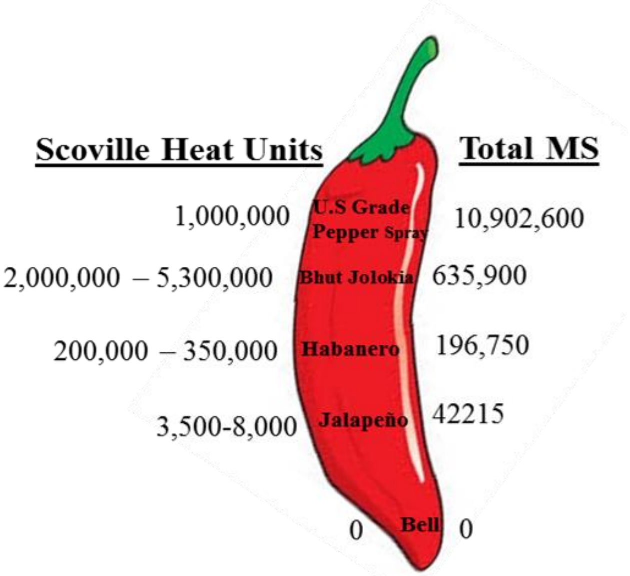
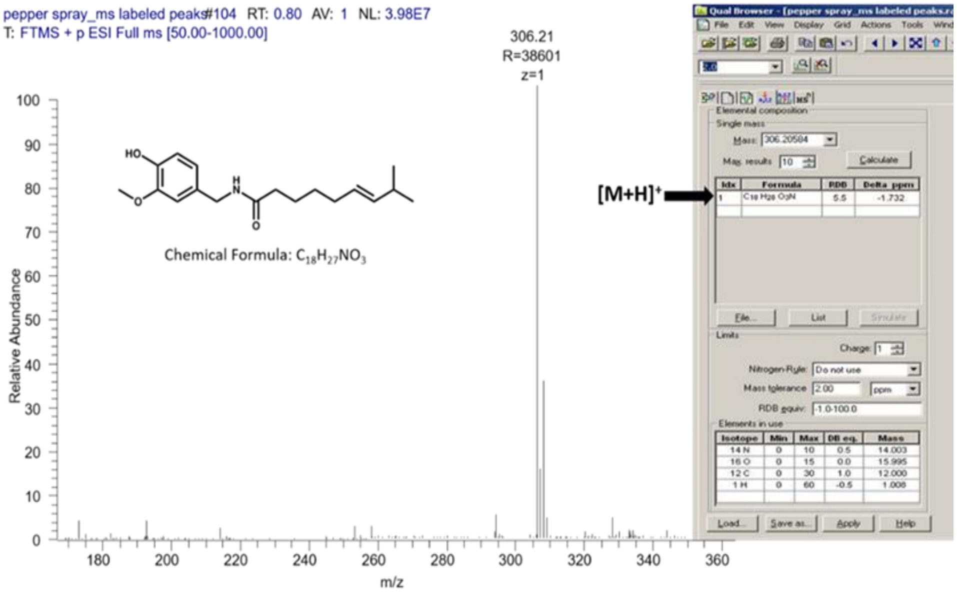


Figure 20. Correlation of thread spray mass spectrometry total capsaicinoids' ion intensities and Scoville Heat Units (SHUs) using various pepper fruits [70]

3.2.4 High Resolution Pepper Fruit Analysis

In recent years, there has been a rise in acquiring accurate mass/identification for components in complex mixtures in food detection using the Orbitrap mass analyzer [71]. The Orbitrap mass analyzer allows for high mass accuracy that can immediately determine elemental composition of an analyte of choice [72]. To determine the accuracy of the thread spray ionization method and its multiplex ability, high resolution mass measurements were conducted using habañero pepper fruit sample. The capsaicinoid content in habañero peppers were analyzed using Thermo Fisher Scientific LTQ Orbitrap and results are shown in Figures 21-25. Pepper preparation was similar to previously describes, where one cotton:polyester 35/65 thread was suspended on a needle. By pushing the needle through the habañero capsaicinoids residues deposited onto the thread, which were directly analyzed.



Mass: 306.20584

Max results: 10

Idx	Formula	RDB	Delta ppm
1	$C_{18}H_{28}O_3N$	5.5	-1.732

Charge: 1

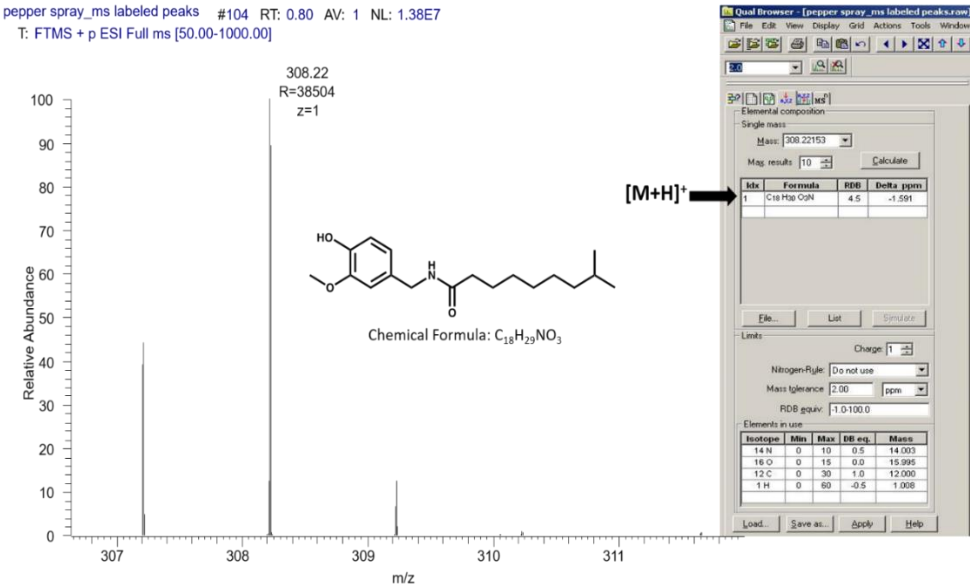
Nitrogen-Rule: Do not use

Mass tolerance: 2.00 ppm

RDB equiv: -1.0-100.0

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	0	30	1.0	12.000
1 H	0	60	-0.5	1.008

Figure 21. Exact mass analysis of capsaicin, *MW* 305, using thread spray Orbitrap mass spectrometer. Error is -1.732 ppm



Mass: 308.22153

Max results: 10

Idx	Formula	RDB	Delta ppm
1	$C_{18}H_{30}O_3N$	4.5	-1.591

Charge: 1

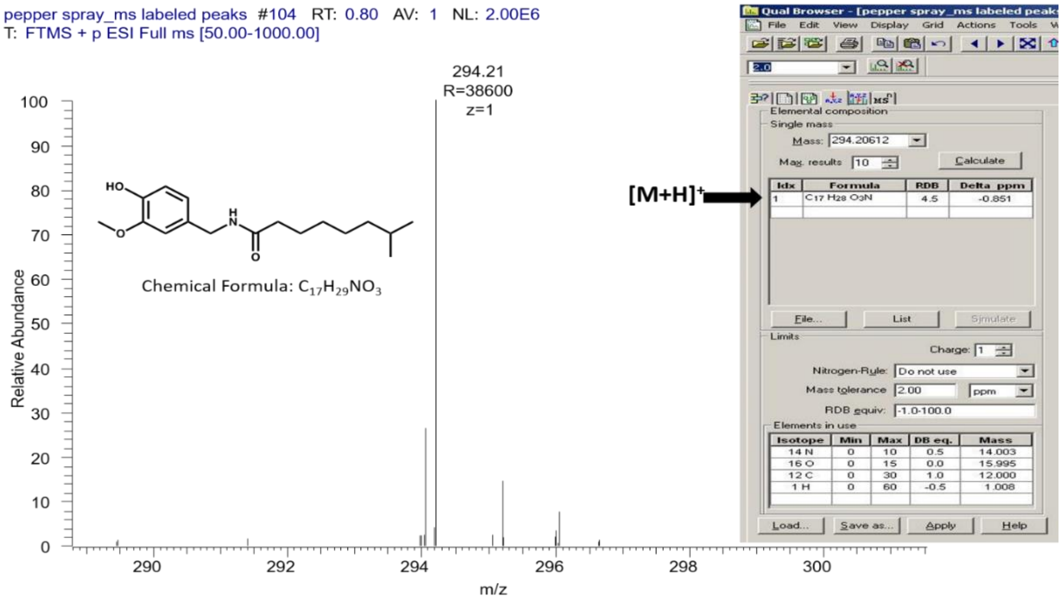
Nitrogen-Rule: Do not use

Mass tolerance: 2.00 ppm

RDB equiv: -1.0-100.0

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	0	30	1.0	12.000
1 H	0	60	-0.5	1.008

Figure 22. Exact mass analysis of dihydrocapsaicin, *MW* 307, using thread spray Orbitrap mass spectrometer. Error is -1.591 ppm



Mass: 294.20612

Max. results 10

Idx	Formula	RDB	Delta ppm
1	C17 H28 O3N	4.5	-0.851

Charge: 1

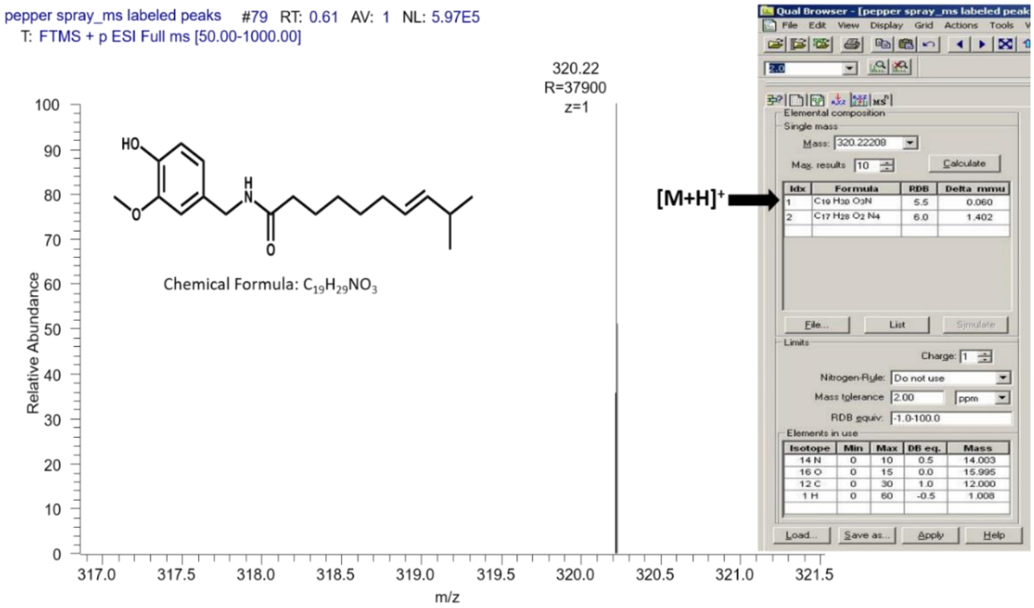
Nitrogen-Rule: Do not use

Mass tolerance 2.00 ppm

RDB equiv: -1.0-100.0

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	0	30	1.0	12.000
1 H	0	60	-0.5	1.008

Figure 23. Exact mass analysis of nordihydrocapsaicin, *MW* 294, using thread spray Orbitrap mass spectrometer. Error is -0.851 ppm



Mass: 320.22208

Max. results 10

Idx	Formula	RDB	Delta mmu
1	C19 H30 O3N	5.5	0.060
2	C17 H28 O2 N4	6.0	1.402

Charge: 1

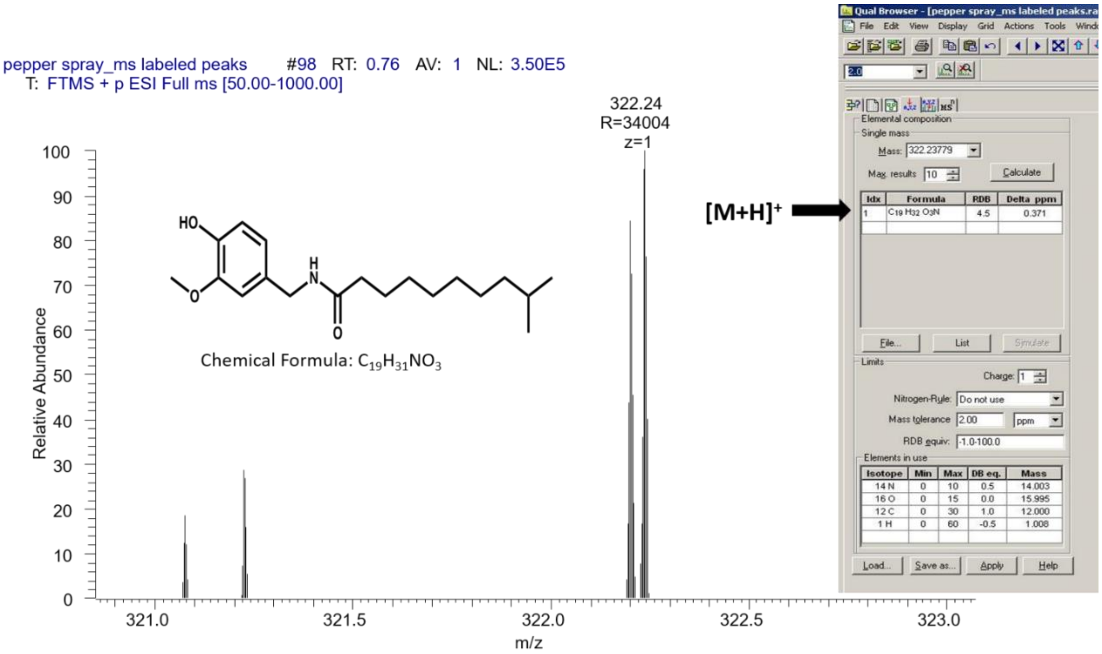
Nitrogen-Rule: Do not use

Mass tolerance 2.00 ppm

RDB equiv: -1.0-100.0

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	0	30	1.0	12.000
1 H	0	60	-0.5	1.008

Figure 24. Exact mass analysis of homocapsaicin, *MW* 319, using thread spray Orbitrap mass spectrometer. Error is 0.060 ppm.



2.0

Mass: 322.23779

Max. results 10

Idx	Formula	RDB	Delta ppm
1	$C_{19}H_{32}O_3N$	4.5	0.371

Charge: 1

Nitrogen-Rule: Do not use

Mass tolerance 2.00 ppm

RDB equiv: -1.0-100.0

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	0	30	1.0	12.000
1 H	0	60	-0.5	1.008

Figure 25. Exact mass analysis of homodihydrocapsaicin, *MW* 322, using thread spray Orbitrap mass spectrometer. Error is 0.371 ppm

Exact mass analysis determined the ions generated from the thread spay ionization method are the expected capsaicinoids from the habañero pepper fruit. Corresponding errors associated with the exact mass measurement are also provided, which was less than 2 ppm in all cases. These results prove that thread spray mass spectrometry is a reliable and versatile method for direct analysis of capsaicinoids in pepper fruits.

Chapter 4 Conclusion and Future Work

4.1 Conclusion

Thread spray ionization has been demonstrated in this study to be an effective method for sampling the interior of pepper fruits and the analysis of pepper spray residues on clothing with minimal destruction. Threads from four major off-the-shelf fabrics cotton:polyester (35/65), 100% cotton, 100% polyester, and nylon were tested and found to be effective in ionization capsaicinoids from complex pepper products. The sharp tips of the threads provided a direct means to perform substrate-based ambient ionization, which could be interfaced with various mass spectrometers. The thread spray mass spectrometry method could be used in the food industry for routine qualitative and quantitative analysis of capsaicinoids in pepper and in forensic applications for the detection of pepper spray residues, all requiring no sample pre-treatment. The successful use of threads as sampling and ionization media simplifies mass spectrometric-based analytical method development and provides rapid, inexpensive, and in-situ analysis. Other features such as the elimination of nebulizing gas and external pumps are attractive for field analysis using hand-held mass spectrometers.

4.2 Future Work

For future work, the goal is to show that thread spray ionization source has the ability to be coupled to a mass spectrometer and rapid identification of a simple protein. To do this there are two main goals to accomplish this larger goal. The first is to develop a thread spray source adapter that allows continuous solvent flow in the apparatus. The second is change the surface of different threads in order to create a reliable stationary phase in order to get separation and characterizing of proteins.

References

(1) Chowański, S.; Adamski, Z.; Marciniak, P.; Rosiński, G.; Büyükgüzel, E.; Büyükgüzel, K.; Falabella, P.; Scrano, L.; Ventrella, E.; Lelario, F.; Bufo, S. A. *Toxins* **2016**, *8* (3).

(2) Perry, L.; Dickau, R.; Zarrillo, S.; Holst, I.; Pearsall, D. M.; Piperno, D. R.; Berman, M. J.; Cooke, R. G.; Rademaker, K.; Ranere, A. J.; Raymond, J. S.; Sandweiss, D. H.; Scaramelli, F.; Tarble, K.; Zeidler, J. A. *Science* **2007**, *315* (5814), 986–988.

(3) Altman, R. D.; Aven, A.; Holmburg, C. E.; Pfeifer, L. M.; Sack, M.; Young, G. T. *Semin. Arthritis Rheum.* **1994**, *23* (6), 25–33.

(4) Hunter, J. *FBI Law Enforcement Bulletin* **1994**, 24–26.

(5) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389* (6653), 816–824.

(6) Caterina, M. J. *Science* **2000**, *288* (5464), 306–313.

(7) Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51* (2), 159–212.

(8) Watson, W. A.; Stremel, K. R.; Westdorp, E. J. *Ann. Pharmacother.* **1996**, *30* (7–8), 733–735.

(9) Lee, R. J.; Yolton, R. L.; Yolton, D. P.; Schnider, C.; Janin, M. L. *J. Am. Optom. Assoc.* **1996**, *67* (9), 548–560.

(10) Busker, R. W.; van Helden, H. P. *Am. J. Forensic Med. Pathol.* **1998**, *19* (4), 309–316.

(11) Ellis, C. N.; Berberian, B.; Sulica, V. I.; Dodd, W. A.; Jarratt, M. T.; Katz, H. I.; Prawer, S.; Krueger, G.; Rex, I. H.; Wolf, J. E. *J. Am. Acad. Dermatol.* **1993**, *29* (3), 438–442.

(12) Peter, K. V. *Handbook of Herbs and Spices*; Elsevier, 2012.

(13) Scoville, W. L. *J. Am. Pharm. Assoc. 1912* **1912**, *1* (5), 453–454.

(14) Bajaj, K. L. *J. Assn. Offic. Anal. Chem.* **1980**, *63*, 1314–1316.

(15) Palacio, J. J. . **1979**, *62* (5), 1165–1170.

(16) Gibbs, H. A. .; O'Garro, L. . *HORTSCIENCE* **2004**, *39*, 132–135.

(17) Constant, H. L.; Cordell, G. A.; West, D. P.; Johnson, J. H. *J. Nat. Prod.* **1995**, *58* (12), 1925–1928.

(18) Jarret, R.; Perkins, B.; Fan, T.; Prince, A.; Guthrie, K.; Skoczenski, B. *2003 16*, 189–194.

(19) Krajewska, A. M.; Powers, J. J. *J. Chromatogr. A* **1988**, *457*, 279–286.

(20) Iwai, K.; Suzuki, T.; Fujiwake, H.; Oka, S. *J. Chromatogr.* **1979**, *172*, 303–311.

(21) Reilly, C. A.; Crouch, D. J.; Yost, G. S. *J. Forensic Sci.* **2001**, *46* (3), 502–509.

(22) Reilly, C. A.; Crouch, D. J.; Yost, G. S.; Fatah, A. A. *J. Chromatogr. A* **2001**, *912* (2), 259–267.

(23) Badu-Tawiah, A. K.; Eberlin, L. S.; Ouyang, Z.; Cooks, R. G. *Annu. Rev. Phys. Chem.* **2013**, *64*, 481–505.
(24) Monge, M. E.; Harris, G. A.; Dwivedi, P.; Fernández, F. M. *Chem. Rev.* **2013**, *113* (4), 2269–2308.
(25) Huang, M.-Z.; Yuan, C.-H.; Cheng, S.-C.; Cho, Y.-T.; Shiea, J. *Annu. Rev. Anal. Chem.* **2010**, *3* (1), 43–65.
(26) Takats, Z. *Science* **2004**, *306* (5695), 471–473.
(27) Badu-Tawiah, A.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2010**, *21* (8), 1423–1431.
(28) Badu-Tawiah, A.; Bland, C.; Campbell, D. I.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2010**, *21* (4), 572–579.
(29) Cody, R. B.; Laramée, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77* (8), 2297–2302.
(30) Andrade, F. J.; Shelley, J. T.; Wetzel, W. C.; Webb, M. R.; Gamez, G.; Ray, S. J.; Hieftje, G. M. *Anal. Chem.* **2008**, *80* (8), 2646–2653.
(31) Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Zhang, X.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2008**, *80* (23), 9097–9104.
(32) Cheng, S.-C.; Cheng, T.-L.; Chang, H.-C.; Shiea, J. *Anal. Chem.* **2009**, *81* (3), 868–874.
(33) Nemes, P.; Vertes, A. *Anal. Chem.* **2007**, *79* (21), 8098–8106.
(34) Rezenom, Y. H.; Dong, J.; Murray, K. K. *Analyst* **2008**, *133* (2), 226–232.
(35) Chen, H.; Ouyang, Z.; Cooks, R. G. *Angew. Chem. Int. Ed.* **2006**, *45* (22), 3656–3660.
(36) Snyder, D. T.; Pulliam, C. J.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2016**, *88* (1), 2–29.
(37) Hendricks, P. I.; Dalgleish, J. K.; Shelley, J. T.; Kirleis, M. A.; McNicholas, M. T.; Li, L.; Chen, T.-C.; Chen, C.-H.; Duncan, J. S.; Boudreau, F.; Noll, R. J.; Denton, J. P.; Roach, T. A.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2014**, *86* (6), 2900–2908.
(38) Zhai, Y.; Feng, Y.; Wei, Y.; Wang, Y.; Xu, W. *The Analyst* **2015**, *140* (10), 3406–3414.
(39) Kumano, S.; Sugiyama, M.; Yamada, M.; Nishimura, K.; Hasegawa, H.; Morokuma, H.; Inoue, H.; Hashimoto, Y. *Mass Spectrom. Tokyo Jpn.* **2015**, *4* (1), A0038.
(40) Bokhart, M. T.; Muddiman, D. C. *Analyst* **2016**, *141* (18), 5236–5245.
(41) Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem. Int. Ed.* **2010**, *49* (5), 877–880.
(42) Damon, D. E.; Davis, K. M.; Moreira, C. R.; Capone, P.; Cruttenden, R.; Badu-Tawiah, A. K. *Anal. Chem.* **2016**, *88* (3), 1878–1884.

(43) Damon, D. E.; Maher, Y. S.; Yin, M.; Jjunju, F. P. M.; Young, I. S.; Taylor, S.; Maher, S.; Badu-Tawiah, A. K. *Analyst* **2016**, *141* (12), 3866–3873.
(44) Chen, S.; Wan, Q.; Badu-Tawiah, A. K. *J. Am. Chem. Soc.* **2016**, *138* (20), 6356–6359.
(45) Gómez-Ríos, G. A.; Pawliszyn, J. *Angew. Chem. Int. Ed.* **2014**, *53* (52), 14503–14507.
(46) Yang, Y.; Deng, J.; Yao, Z.-P. *J. Am. Soc. Mass Spectrom.* **2014**, *25* (1), 37–47.
(47) Zhang, Z.-P.; Liu, X.-N.; Zheng, Y.-J. *Chin. J. Anal. Chem.* **2014**, *42* (1), 145–152.
(48) Http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_51541.pdf
(49) Thermo Fisher :: Orbitrap :: LTQ Orbitrap XL http://planetorbitrap.com/ltq-orbitrap-xl#.WUHpkOmQzIU (accessed Jun 15, 2017).
(50) VK-X Series 3D Laser Scanning Confocal Microscope | KEYENCE http://www.keyence.com/ss/products/microscope/vkx/ (accessed Jun 17, 2017).
(51) Gross, J. H. *Mass Spectrometry*; Springer Berlin Heidelberg: Berlin, Heidelberg, 2011.
(52) Todd, J. F. J. *Int. J. Mass Spectrom* **1995**, *142*, 211–240.
(53) Price, P. *J. Am. Soc. Mass Spectrom.* **1991**, No. 2, 336–348.
(54) Ren, Y.; Wang, H.; Liu, J.; Zhang, Z.; McLuckey, M. N.; Ouyang, Z. *Chromatographia* **2013**, *76* (19–20), 1339–1346.
(55) Espy, R. D.; Muliadi, A. R.; Ouyang, Z.; Cooks, R. G. *Int. J. Mass Spectrom.* **2012**, *325–327*, 167–171.
(56) Page, J. S.; Kelly, R. T.; Tang, K.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **2007**, *18* (9), 1582–1590.
(57) Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G. *Analyst* **2010**, *135* (4), 669–681.
(58) Li, A.; Wang, H.; Ouyang, Z.; Cooks, R. G. *Chem. Commun.* **2011**, *47*, 2811.
(59) Kiontke, A.; Oliveira-Birkmeier, A.; Opitz, A.; Birkemeyer, C. *PLoS ONE* **2016**, *11* (12).
(60) Liu, J.; Wang, H.; Manicke, N. E.; Lin, J.-M.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2010**, *82* (6), 2463–2471.
(61) Chinn, M. S.; Sharma-Shivappa, R. R.; Cotter, J. L. *Food Bioprod. Process.* **2011**, *89* (4), 340–345.
(62) Bhushan, B. In *Modern Tribology Handbook, Vol. 1 - Principles of Tribology*; CRC Press: Boca Raton, Florida; Vol. 2.
(63) Douglas, J. F. *Macromolecules* **1989**, *22* (9), 3707–3716.
(64) Moita, A. S.; Moreira, A. L. *Zaragoza* **2002**, *9* (11).
(65) Cordell, G. A.; Araujo, O. E. *Ann. Pharmacother.* **1993**, *27* (3), 330–336.
(66) Kopec, S. E.; DeBellis, R. J.; Irwin, R. S. *Pulm. Pharmacol. Ther.* **2002**, *15* (6), 529–534.
(67) Bode, A. M.; Dong, Z. *Cancer Res.* **2011**, *71* (8), 2809–2814.

(68) Alothman, Z. A.; Wabaidur, S. M.; Khan, M. R.; Ghafar, A. A.; Habila, M. A.; Ahmed, Y. B. H. *J. Sep. Sci.* **2012**, *35* (21), 2892–2896.
(69) Usman, M. G.; Rafii, M. Y.; Ismail, M. R.; Malek, M. A.; Latif, M. A. *Mol. Basel Switz.* **2014**, *19* (5), 6474–6488.
(70) GRINGO HOT CHILE PEPPER GUIDE http://www.heliotricity.com/hotchilepepperguide.html (accessed Jun 18, 2017).
(71) Makarov, A.; Scigelova, M. *J. Chromatogr. A* **2010**, *1217* (25), 3938–3945.
(72) Makarov, A.; Denisov, E.; Kholomeev, A.; Balschun, W.; Lange, O.; Strupat, K.; Horning, S. *Anal. Chem.* **2006**, *78* (7), 2113–2120.