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Forensic Identification of Condom Lubricants by Advanced Statistical Analysis Processing of Direct Analysis in Real Time Mass Spectrometric Data – Towards Development of a Condom Residue Database for Forensic Science Practitioners

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ABSTRACT

The ability to identify condom derived trace evidence is gaining importance because of the increasing frequency with which perpetrators of sexual assault use condoms in order to avoid leaving behind incriminating DNA evidence that might link them to the crime. Although DNA remains the gold standard for sexual assault evidence, other forms of trace evidence are needed in its absence. When condoms are used, the lubricants associated with them are the trace evidence. For the lubricant residue to be useful in a forensics context, a database of chemical signatures of lubricants against which acquired evidence can be screened is required, so that condom brands and types can be identified from the trace evidence. Towards the goal of developing such a database, this study used direct analysis in real time-high resolution mass spectrometry (DART-HRMS) to analyze 110 different types of condoms representing 16 brands. Over 700 spectra were acquired, each serving as a chemical fingerprint for the analyzed condom. The results showed that condoms of the same type within different brands exhibited the same chemical signatures, which differed from condoms of other brands, even when the condoms were advertised to have the same characteristics. For example, the mint flavored condoms of different brands had distinct diagnostic chemical signatures that allowed them to be distinguished from one another, even though they all contained the same chemical components that conferred the mint flavor. Both supervised and unsupervised multivariate statistical analyses were performed on the data. Hierarchical clustering analysis (unsupervised) showed that condoms could be differentiated by brand. Kernel discriminant analysis (supervised) showed that condoms within a given brand could be distinguished. The observed leave-one-out cross validation was 90-100% depending upon the brand. This indicates that a strong database has been developed with the capability of serving as a presumptive test that can be used not only to identify brands, but also the particular condom type within a brand. This database can be readily expanded as additional condom types emerge, and may be particularly useful for corroborating the accounts of victims, or exonerating the falsely accused in cases where DNA evidence is lacking. The positive brand identification of unknown condom residues implies that the database could serve as a tool to assist forensic science practitioners in prosecuting sexual assault cases.

Acknowledgements

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Introduction

Each year, over three hundred and twenty thousand Americans aged twelve and over are sexually assaulted or raped, and ninety percent of these victims are women [1,2]. Typically, the evidence used to identify the perpetrator is DNA collected from a rape test kit. Unfortunately, for a variety of reasons including the absence of dedicated time and resources for kit analysis, outdated lab policies, and the considerable expense associated with kit analysis, there is an abundance of rape test kits that have yet to be tested. This "backlog" includes cases that are over twenty years old, and it is estimated that there are approximately one hundred and seventy five thousand untested kits in labs across the United State alone [3,4]. By and large, the most valuable substance that can be collected with a rape kit is DNA, as it can establish a definitive link between a victim and the assailant. However, the increasing awareness that perpetrators of sexual assault have of the power of DNA has resulted in the increasing use of condoms, in order to avoid leaving behind incriminating biological evidence. In the absence of DNA, it is especially difficult to establish a link between a suspect and a victim. For this reason, there has been increased interest in alternative forms of trace evidence, such as residue left behind if a condom was used in the course of the assault.

The earliest studies of condom lubricants as trace evidence sought to establish means by which to confirm the presence of condom constituents at the crime scene or on the body of the victim. These reports focused on primary condom components such as the slip agent polydimethylsiloxane (PDMS). This molecule was first detected in the context of a sexual assault using Fourier transform infrared spectroscopy (FT-IR) and desorption chemical ionization mass spectrometry (DCI-MS) [5]. Fourier self-deconvolution (FSD) was then used as a method for identification of PDMS [6]. Other components commonly found in condom lubricants for which

methods of detection have been developed include the spermicide nonoxynol-9 (N-9) and polyethylene glycol (PEG). These species have been detected using liquid chromatography mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectroscopy (DRIFTS), gas chromatography mass spectrometry (GC-MS), Raman spectroscopy, micellar electrokinetic capillary chromatography (MEKC), matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS), direct analysis in real time-high resolution mass spectrometry (DART-HRMS), and desorption electrospray ionization mass spectrometry (DESI-MS) [7-21].

In cases of sexual assault, biological fluids will most likely be found on the swab of the rape test kit used to examine the victim. Blackledge and Vincenti found that both vaginal and seminal secretions do not interfere with the identification of PDMS [8]. It has also been observed that any condom lubricant that is found within the biological sample will not interfere with the analysis of DNA when FT-Raman spectroscopy is used to perform the analysis [13]. Studies have also been conducted to determine the lifetimes of condom-derived compounds when they are combined with biological fluids, in order to determine how long the compounds survive and remain detectable. PDMS was detected at 12 hours post-intercourse and was found to last for a minimum of 24 hours when analyzed by FT-IR [8, 5]. When pyrolysis GC-MS is used, the PDMS is converted to cyclic dimethyl siloxanes (DMS) which were detected in large recoverable amounts for up to 12 hours post intercourse [10]. When using MEKC however, no lubricant could be detected after 30 minutes when the samples were acquired from skin or contaminated cloth [14].

Fingerprints are a commonly used identification tool in criminal cases. Exploiting the technique of imaging mass spectrometry, MALDI-MS was used to visualize condom lubricant-

laden prints, where the image was based on the presence of compounds peculiar to the lubricant. The importance of this finding is that it provides a means whereby a perpetrator can not only be identified from their print, but it can also be established that they had contact with a condom. A drawback with MALDI-MS though, is that a specific matrix must be identified to ensure that the biological fluids do not interfere [16]. DART-HRMS has been used to analyze fingerprints containing condom lubricants. It was demonstrated using this method that lubricant could be detected without the interference of endogenous compounds present in the print [17, 18]. An advantage to DART-HRMS as an analysis approach is that there is no matrix requirement and samples can be analyzed in their native form.

It has also been proposed that condom differentiation can be accomplished based on differences between brands in the viscosity of the PDMS used in the manufacture of the lubricant [6]. Based on this, protocols were developed to individualize various condoms [8, 9]. However, the approach was impractical and difficult to integrate into the routine of crime lab workflows because the analysis involved multiple steps and analytical techniques. MEKC was used to correctly classify 233 out of 263 condoms and personal lubricants, but since this method is not compatible with biological fluids, it may not be useful in a forensic application [14]. MALDI-MS, DART-HRMS, and DESI-MS have been used to test small sample sets of condom lubricants and classify them into various subgroups [16-21] based on multivariate statistical analysis processing of the data.

Currently, identification of lubricant evidence relies primarily on visual comparison and interpretation of spectroscopic or mass spectrometric data. However, for the interpretation of this type of evidence to be less subjective and less prone to human observer bias and errors, it is necessary that a more objective means of interpretation be implemented. Thus, a number of

investigations have focused on the application of multivariate statistical analysis to the processing of condom fingerprint data [14, 16, 17, 19, 20, 21]. For a limited number of samples, these studies have demonstrated proof-of-principle that high condom identity prediction accuracy rates can be achieved through the application of statistical analysis processing of condom chemical signature data. However, there has yet to be a study of a broad range of brands and types of condoms with the purpose of determining features distinctive to a condom at the level at which the brand *and* type can be ascertained. Since sexual assaults and other related crimes such as international trafficking are not limited to the United States and instead are global in scope, it is important to consider condom brands prevalent in other areas of the world.

Overall, a survey of the literature reveals that a number of bottlenecks exist that prevent the more widespread use of condom residue evidence in a forensics context. These include the significant sample preparation steps, the fact that fairly large amounts of sample are required which can be problematic when only trace amounts of evidence are available, and the fact that many of the analytical techniques used, impose restrictions on how readily the large number of sample replicates required to generate a robust statistical analysis database can be acquired. The study described here had three goals. The first was to develop an analysis approach that could in principle be used to rapidly screen rape kit samples to determine whether or not they contain condom lubricant residue. The tool that was used for this part of the study was DART-HRMS. The second was to explore the utility of DART-HRMS to rapidly generate condom residue fingerprints that could be used as a database against which condom residue unknowns can be screened in order to identify them. To build a strong database, the similarity of the chemical signature for a given type of condom from batch to batch is essential, and thus determination of whether this was the case was a major goal. The third was to develop a statistical analysis

approach to rapidly and accurately identify the brand of condom associated with a given residue, and report the result with a statistical level of certainty.

In this study, 110 unique condom types representing 16 brands originating from multiple countries were analyzed. The condoms were distinguished not only by brand, but also included flavored varieties, those containing compounds designed to elicit specific sensory effects (e.g. anesthetics), and those characterized by having special physical characteristics of the latex itself. DART-HRMS was demonstrated to be uniquely suited to rapidly generating the large number of replicates of condom chemical signatures required for the creation of a database against which condom residues could be screened. Using this technique, it was determined that it is possible to glean a tremendous amount of information from the statistical analysis processing of DART-HRMS-derived chemical fingerprints, and that ultimately, condom residues can be attributed to brand with high accuracy.

Materials and Methods

Instrumentation:

An AccuTOF-DART (JEOL USA, Inc, Peabody MA, USA) high resolution mass spectrometer with a resolving power of 6000 FWHM (full width at half maximum) was used for mass measurements. For soft ionization, orifice 1 was set to 20 V and orifice 2 and the ring lens voltages to 5 V. The grid voltage was set to +50 V. The peaks voltage was set to 600 V to detect ions greater than *m/z* 60. The DART simplified voltage and pressure (SVP) ion source (IonSense Inc., Saugus, MA, USA) was operated using a helium gas heater with temperature set at 350 °C and a flow of 2 L/min. All experiments were conducted in positive ion mode.

Materials:

Condoms were purchased from Amazon (http://www.amazon.com, USA), Wal-Mart (Albany, NY, USA), and JingDong Mall (http://www.jd.com, China). A total of one-hundred and ten different condom types were tested, representing sixteen brands including Aoni, Atlas, Caution Wear, Crown, Durex, Fantasy, Glyde, Jissbon, Kimono, LifeStyles, Mates, Now, Okamoto, One, Pasante and Trojan. These brands were manufactured in the United States, United Kingdom, Australia, Japan, and China.

Methods:

Condom lubricants were tested by swiping the closed end of a melting point capillary tube (VWR, Radnor, PA, USA) on the outer surface of a rolled condom and suspending the tube in the space between the DART ion source and the mass spectrometer inlet for between five and ten seconds. Analyses were performed in replicates of five or ten. All condoms were analyzed in the same manner. Polyethylene glycol (PEG 600) was analyzed with every acquired mass spectrum as a standard for accurate mass determinations.

Multivariate Statistical Analysis:

TSSPro3 software (Shrader Analytical, Detroit, MI, USA) was used for data processing of mass spectra including mass spectral calibration, averaging, background subtraction, and peak centroiding. Mass Mountaineer (RBC Software, Portsmouth, NH, available from http://mass-spec-software.com) was used for mass spectral analyses and some of the statistical analyses, including kernel discriminate analysis. Mass Mountaineer was also used to render the mass spectra as heat maps which were exported into Cluster 3.0 and Java Treeview (Stanford University) for hierarchical clustering analysis. BioNumerics (Applied Maths, Inc, Austin, TX, USA) was also used for hierarchical clustering analysis and generation of circular dendrograms.

Results and Discussion

In this study, DART-HRMS was used to analyze condom-derived lubricants. Table 1 lists the brands and the types of condoms within each brand that were studied.

Table 1. Brands and types of condoms studied along with their lot numbers.

Condom Brand	Condom Type	Lot Number	Condom Brand	Condom Type	Lot Number
Aoni			**Fantasy**		
	Extra Smooth Ultrathin 001	D15AE21210		Lubricated	5044
	Nanosilver Ultrathin 001	D15AE20704		Mint	13F2248
	Overtime	D15AE10501			16F728
	Ultrathin	D15AE20703		Strawberry	16F729
Atlas				Vanilla	16F730
	Blue	16N762	**Glyde**		
	Extra Large	16N760		Blueberry	BB22551
	Non-Lubricated	15X4272		Cola	BL21131
	Purple	16N762		Strawberry	PS20521
	Red	16N762		Vanilla	PV23801
	Studded	15N2886		Wildberry	PW23801
	True Fit	15N1970	**Jissbon**		
	Ultra-Lubed	16N758		Super Moist	JP150610
	Ultra Thin	15N2151		Ultra Thin	JB150618
		15SDPN514		Zero	1507ZCRL91
	Yellow	16N762	**Kimono**		
Caution Wear				Micro Thin	50166-9
	Black Ice	UT27322		Micro Thin Large	50168-9
	Classic	PN27325		Micro Thin plus Aqua Lube	50451-9
	Mission 707	DN21332		Ribbed + Sensi Dots	40554-9
	Wild Rose	RN27322		Thin	50461-9
Crown			**LifeStyles**		
	Skinless Skin	T455		Red	1505991922
Durex				SKYN Extra Lube	1505P10622
	Extra Sensitive	1000079540		SKYN Extra Studded	1511843316
		1000092687		SKYN Original	1507P10722
	Love	1000106912		Ultra Sensitive	1507130416
	Tropical Apple	15F4190A		Ultra Thin	1505020422
	Tropical Banana	15F4190B		Yellow	1505991922
	Tropical Strawberry	15F4190S	**Mates**		
Fantasy				Banana	1412341216
	Banana	16F725		Mint	1410421216
	Chocolate	16F726		Strawberry	1411751216
	Grape	16F727		Vanilla	1411741316

Now			**One**		
	Carnival Banana	1404004-4		Lavender	16N950L
	Carnival Mint	1404004-3		Mint Chocolate	15F1205
	Roller Coaster	1404002		Orange	16N950O
	Speed Bumps	1404003		Pleasure Dome	15N1673
	Super Fine	1404001		Pleasure Plus	14N2282
					14N2284
Okamoto				Purple	16N950P
	003 Aloe	HK026B100		Red	16N950R
	003 Hyaluronic Acid	HK016A101		Super Sensitive	15N846
	003 Platinum	HK016C98		Tantric Pleasures Maori	14N1403
	Charm	HK016A38		Tantric Pleasures Titan	14N1402
	Crown	175J1101		Tantric Pleasures Tribal	14N1401
	Roman	145L1101		The Legend	15N1215
	Ultra Smooth	HK015M39		Vanish	15N474
One					15N475
	576 Sensation	15N3752		Yellow	16N950Y
		15N4269		Zero Thin	1171501
	Aqua	14N1357A			1171502
		14N1367A	**Pasante**		
		16N950A		Blueberry Blast	PL5437B
	Banana Split	15F1204		Chocolate Temptation	PL5437C
	Black	14N1357K		Mint Tingle	PL5437G
		14N1367K		Strawberry Crush	PL5437R
		16N950K	**Trojan**		
	Blue	16N950B		ENZ	0T4277X1
	Bubblegum	15F1201		Her Pleasure Sensations	TT5153UZ922
		16F1207		Intense	TT5146UZ811
	Chocolate Strawberry	15F1208		Magnum Ecstasy	TT4302CB
	Classic Select	14N1579		Magnum Lubricated	TT5054XZ523
	Emerald	16N950E		Magnum Thin	TT5251ZZ1216
	Fresh Mint	15F1206		Magnum Warming	TT4121ZZ516
	Glowing Pleasures	15N858		Twisted	TT5104WZ718
	Green	16N950G		Ultra Ribbed	TT5301Y
	Island Punch	15F1197		Ultra Thin	TT5305BZ1101
		15F1203		Warming	TT5157UZ1302

The JEOL AccuTOF mass spectrometer used has millimass unit resolution and is able to detect nanogram quantities of sample. Unlike most of the previously reported condom residue analysis methods, no sample preparation is required and material can be analyzed directly. In addition, standard DART-HRMS operation is performed under soft ionization conditions. Consequently, the peaks in each spectrum represent the protonated forms of the detected molecules. The output of a typical DART-HRMS experiment is illustrated in Figure 1, which shows one spectrum from each of the sixteen brands analyzed. Each panel is a plot of the *m/z* ratio of the protonated form of the un-fragmented molecule, against the relative intensity of the detected ions. Based on the advertised characteristics and molecules reported in the literature as being present in condom lubricants, it was possible to make tentative assignments for several of the high-resolution masses observed in the spectra. For example, the Mates Vanilla spectrum had a mass at *m/z* 153.0552 which is consistent with the presence of vanillin, the molecule primarily responsible for the flavor and odor of vanilla. Fantasy Banana had a mass at *m/z* 131.1072, consistent with the presence of isoamylacetate, the primary flavor and odor constituent in bananas. Pasante Mint Tingle had a mass at *m/z* 151.1113 which is consistent with the presence of carvone, a primary constituent for mint flavoring. Other molecules commonly found in condoms were tentatively identified. For example, Aoni Extra Smooth Ultrathin 001 had a peak with an *m/z* value of 135.1021, consistent with ethoxydiglycol; Atlas Studded had an *m/z* value of 109.0626 consistent with benzyl alcohol; Caution Wear Black Ice had an *m/z* value of 130.1592 consistent with the presence of *n*-octylamine; Crown Skinless Skin had an *m/z* value of 263.2357 consistent with isonox 132 (2, 6-di-*tert*-butyl-4-*sec*-butyl-phenol); Durex Love had an *m/z* value of 281.2524 consistent with linoleic acid; Glyde Cola had an *m/z* value of 174.1332 consistent with acetone anil; Jissbon Ultra Thin had an *m/z* value of 158.1545 consistent with *N*-

N-dibutylformamide; Kimono Micro Thin Large had an *m/z* value of 519.1423 consistent with (tetradecamethyl) cycloheptasiloxane; LifeStyles SKYN Extra Lube had an *m/z* value of 335.2806 consistent with octyl alcohol ethoxylate (n=5); Now Roller Coaster had an *m/z* value of 150.1129 consistent with acetaldehyde; Okamoto 003 Aloe had an *m/z* value of 119.0696 consistent with γ-hydroxyvaleric acid; One Blue had an *m/z* value of 282.2781 consistent with oleamide; and Trojan Magnum Warming had an *m/z* value of 195.1210 consistent with PEG (n=4). In all, 5 to 10 replicates of the 110 different condom types were measured (i.e. >700 spectra). The full complement of these spectra are in presented in the Appendix.

An important attribute of the types of data that can be classified and thus differentiated using statistical analysis methods is that there is consistency among like samples. To assess this, the DART-HRMS spectra of several samples of the same type of condom but with different lot numbers were compared in head-to-tail plots of their mass spectra. Figure 2 shows the resulting plots for lot number comparisons of Atlas Ultra Thin, Durex Extra Sensitive, Fantasy Mint, and One 576 Sensations. The results show that in every case, the spectra are very similar to one another, indicating that the chemical fingerprints remain consistent between batches.

Numerous types of condoms under various brands are advertised to have the same characteristics. This is particularly true of flavored varieties. For example, brands Fantasy, Mates, Now, One, and Pasante all have mint-flavored condoms. Therefore, the question of whether condoms of different brands but with the same advertised characteristics could be distinguished was investigated. Figure 3 shows a collection of spectra from five different brands, Fantasy, Mates, Now, One, and Pasante, representing six types of condoms that are all advertised to be mint flavored. In each panel, the full mass spectrum is shown in the inset, while the area of the spectrum where the mint flavoring agents carvone and thujone would appear (*m/z* 151.1123

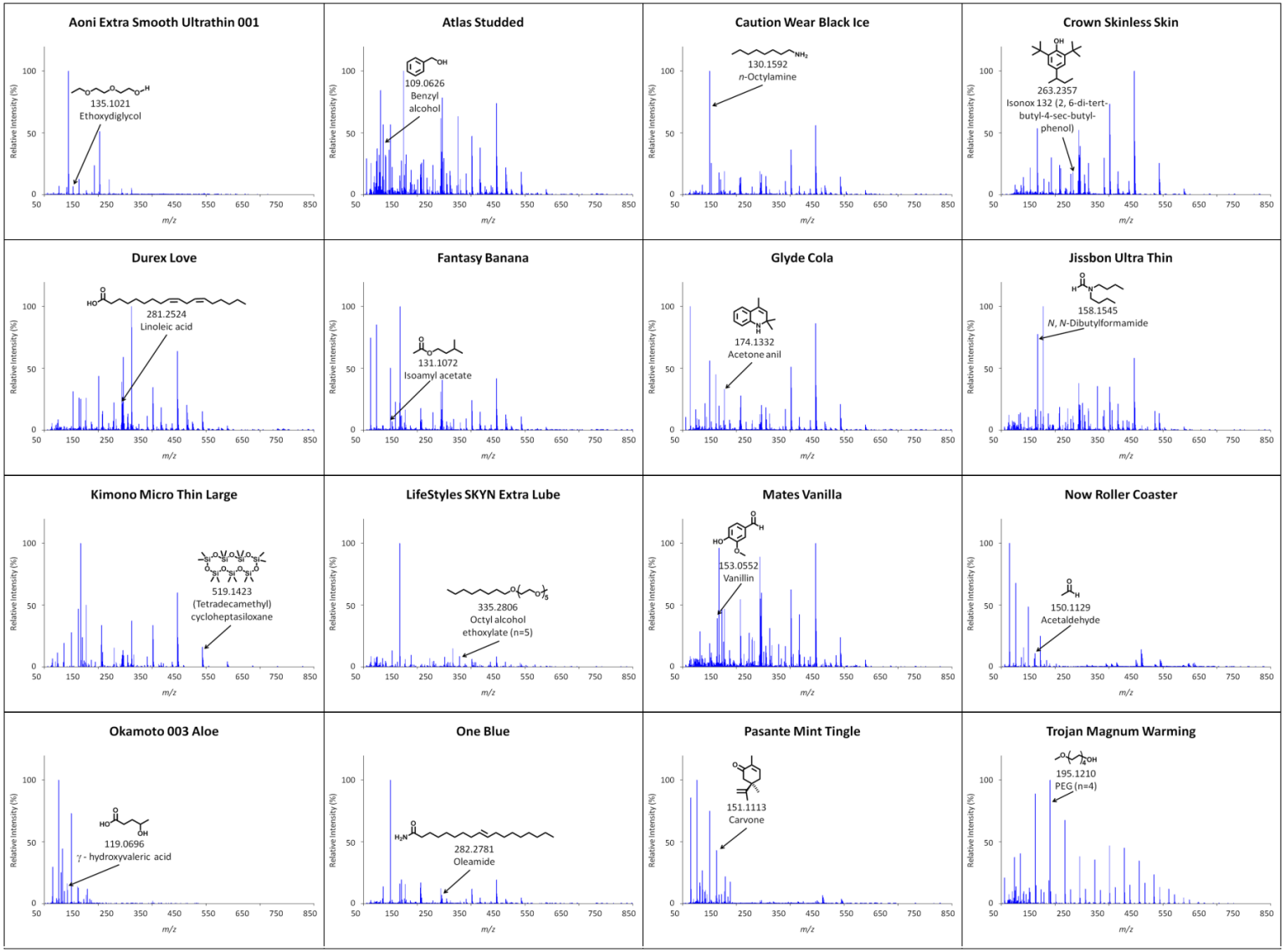

Figure 1. DART-HRMS of condom lubricants. One spectrum for each of the brands studied is presented, and each panel indicates the type of condom represented in the spectrum. The spectra were acquired under soft ionization conditions in positive ion mode. Thus, the peaks represent the protonated forms of the detected molecules. The structures of tentatively identified molecules are shown.

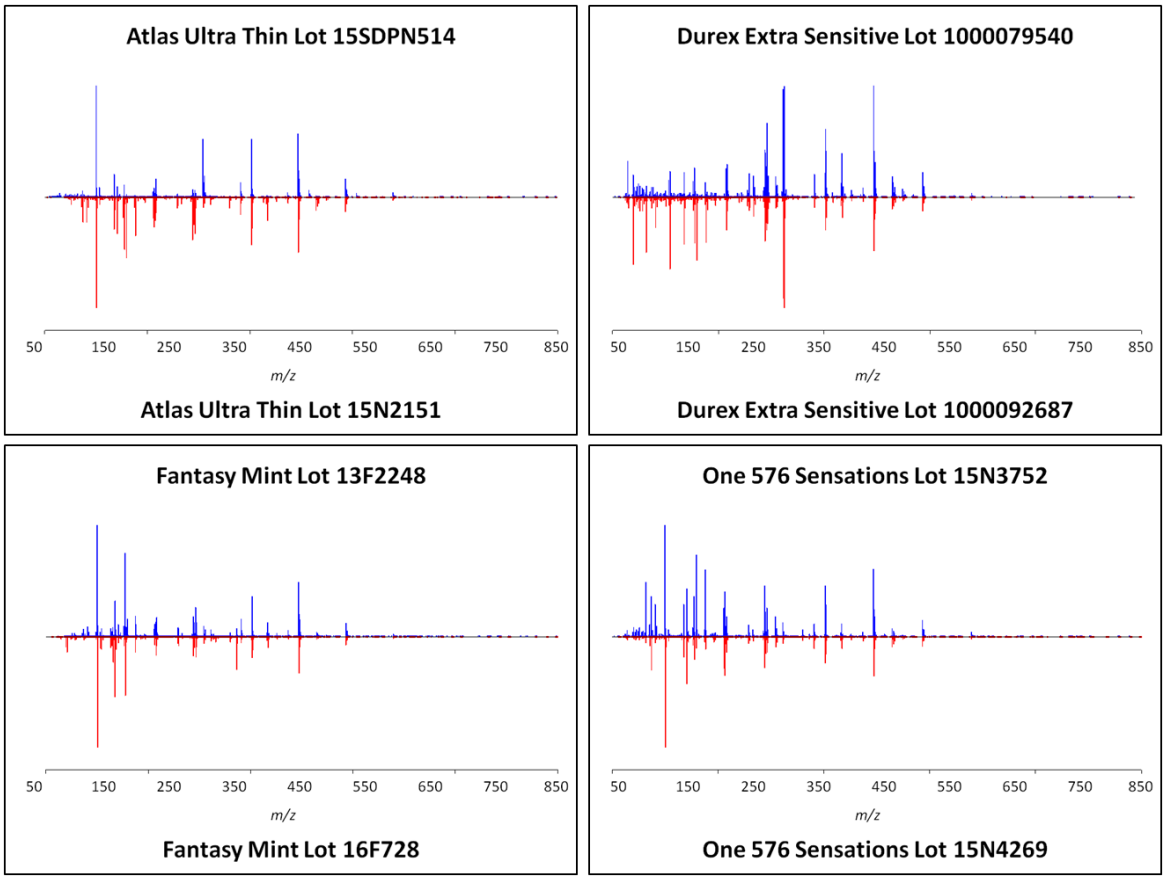

Figure 2. DART-HRMS of Atlas Ultra Thin, Durex Extra Sensitive, Fantasy Mint, and One 576 Sensations condoms, rendered as head-to-tail plots. Each panel shows two spectra from samples with different lot numbers.

and 153.1280 respectively when protonated) is magnified for clarity. Carvone and thujone were identified in the mass spectra of all samples except Now Carnival Mint. However, although there were peaks common to most of the spectra, their overall chemical fingerprints were nevertheless unique. This indicates that even brands marketed as having the same lubricant characteristics have spectra that can be visually distinguished.

Previous studies have shown that a variety of techniques can be used to establish the presence of condom-derived trace evidence through identification of compounds such as PDMS or N-9. However, since numerous condoms across several brands contain these substances, this information in and of itself has limited usefulness. The evidentiary value of condom trace evidence would be enhanced if the type of condom used could be determined. In this regard, a

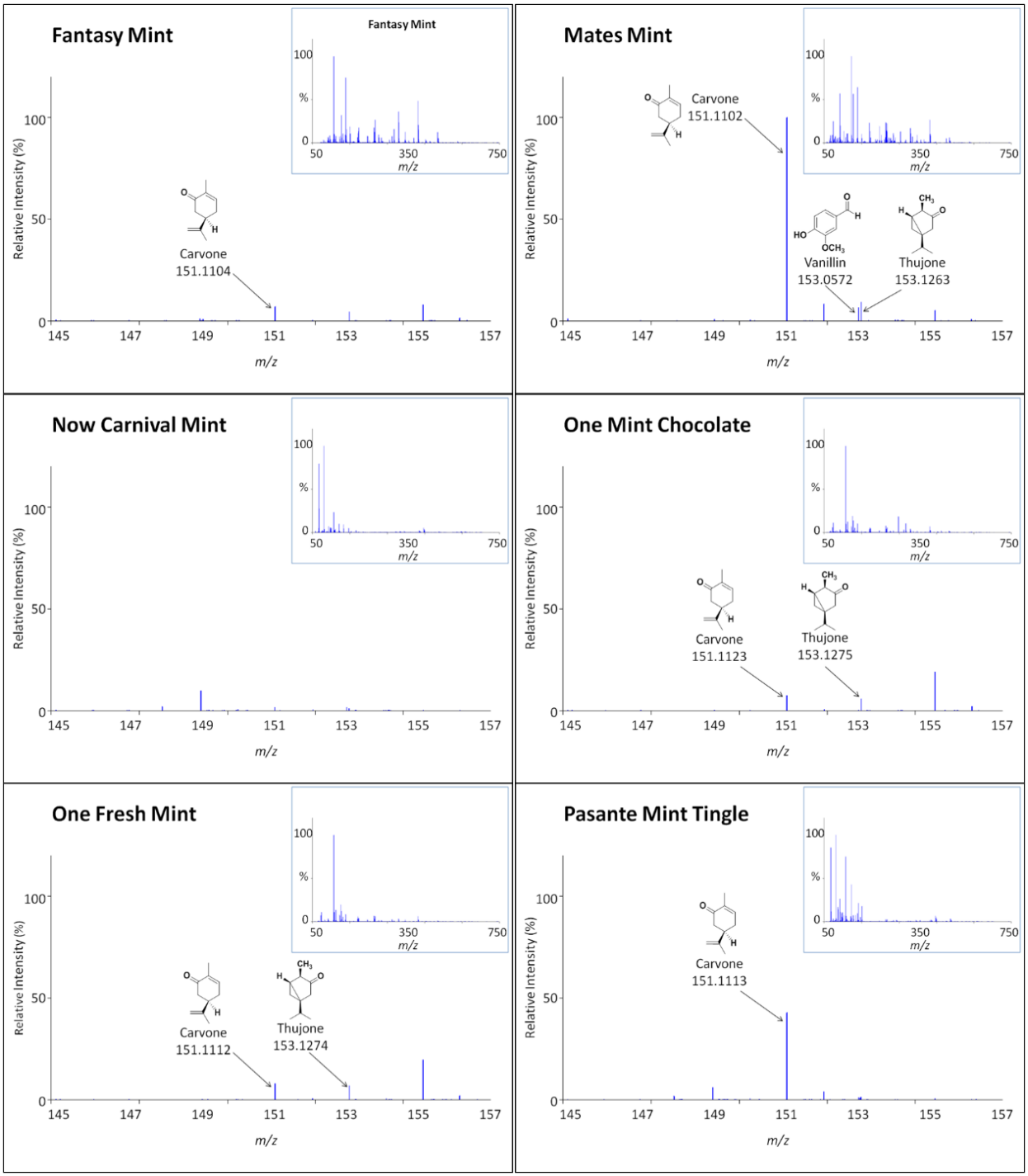

Figure 3. Comparison of the spectra of different brands of condoms all advertised to be mint flavored. In each panel, the area where the high-resolution masses of mint flavor and odor chemicals (i.e. carvone and thujone) appear in magnified, while the full spectrum is shown in the inset. A peak tentatively identified as vanillin, is highlighted in the Mates Mint spectrum.

potential challenge is the possibility that different types of condoms that fall under the same

brand may have the same base lubricant formulation, resulting in chemical fingerprints that are difficult to tell apart. To determine whether condoms of different types but of the same brand can be distinguished, the spectra of the condoms within each brand were compared. Representative examples of this comparison are shown in Figures 4 and 5. In Figure 4, the spectra of four condoms in the Pasante brand are presented, and in Figure 5, the spectra of four condoms in the

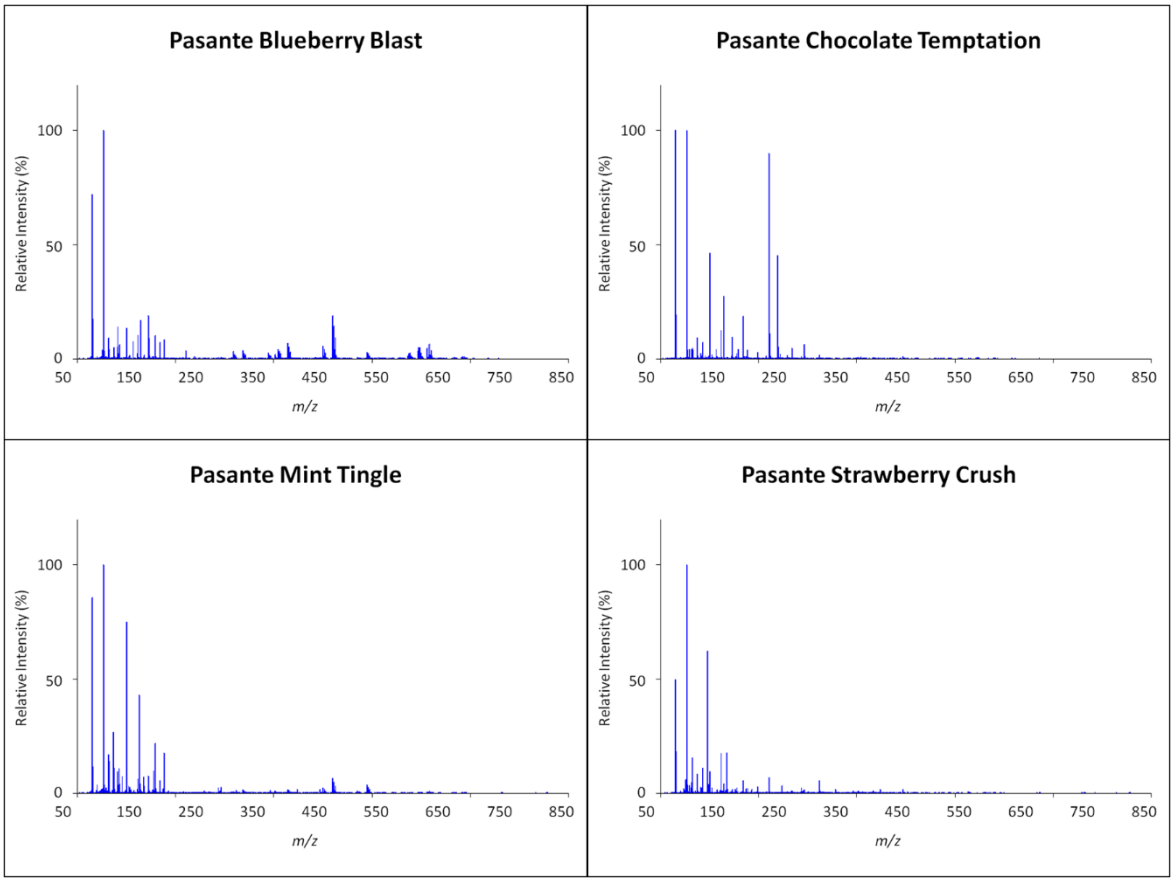

Figure 4. Representative DART-HRMS spectra of four condom types within the brand Pasante.

Aoni brand are shown. All of the spectra have several prominent peaks in common such as the *m/z* values 74.097, 93.056, 130.161 and 149.082 in Pasante, and the *m/z* values 122.097, 198.147, 214.254 and 308.259 in Aoni. Nevertheless, the condoms were visually distinguishable, with the greatest contrast being between condoms advertised to have unique lubricant features such flavors. For example, spectra of the Pasante condoms are visually quite different, which

was not unexpected given that they are advertised to have different flavor characteristics, which presupposes that their chemical makeups would be different. On the other hand, for condoms that differed in the physical features of the latex, such as thin, ribbed, or studded, it was more difficult to visually distinguish between them. This implies that for such condoms, similar or identical base formulas were used, with the primary difference between them being the characteristics of the latex. This may be the case for the Aoni condoms which are much more similar to one another (Figure 5). However, even these condoms were visually distinguishable.

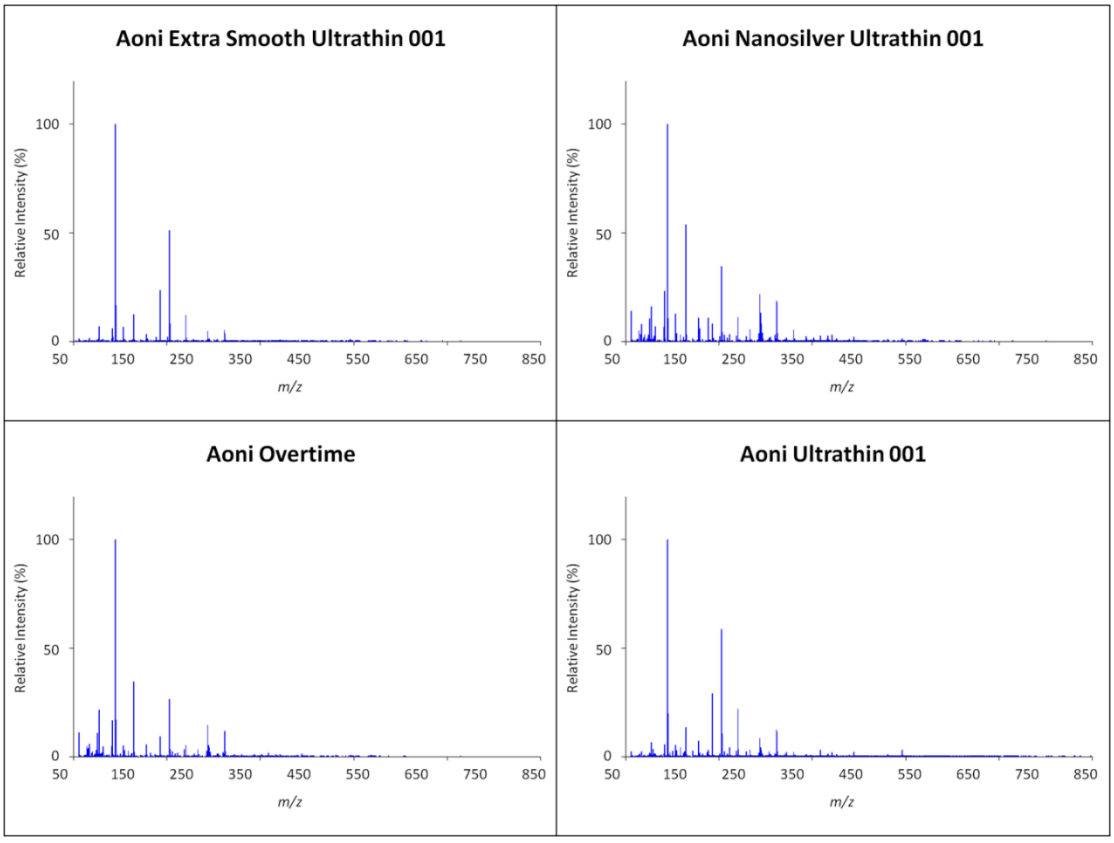

Figure 5. Representative DART-HRMS spectra of four condom types within the brand Aoni.

Multivariate statistical analysis processing of the data was used to determine if this approach would enable the ability to differentiate between and identify condom types within the same brand, and reduce human observer bias in the interpretation of the results. For this, kernel discriminant analysis (KDA) was used. This analysis was accomplished using a subset of feature

masses that were unique to each of the types of condoms which would maximize the ability to separate the condom classes. The subset of feature masses is shown in Table 2. The masses were chosen because they were unique to each condom type. The KDA plot illustrating the results for Fantasy is shown in Figure 6. The clustering of points of the same type and their separation from the others indicates that condoms within a brand can be readily

Table 2. Feature masses used for KDA for condoms in the Fantasy brand.

Mass	Mass
73.0652	308.2568
115.1094	309.2438
130.1589	327.2518
133.0699	356.0764
149.0964	371.1026
158.1536	373.1038
163.1140	374.1049
172.1214	397.3863
174.1312	415.2535
190.1247	429.0929
222.0984	445.1241
223.0642	446.1276
257.2487	447.1199
263.2366	473.1198
280.2608	475.1130
281.2512	519.1413
284.2932	520.1472
285.2793	521.1416

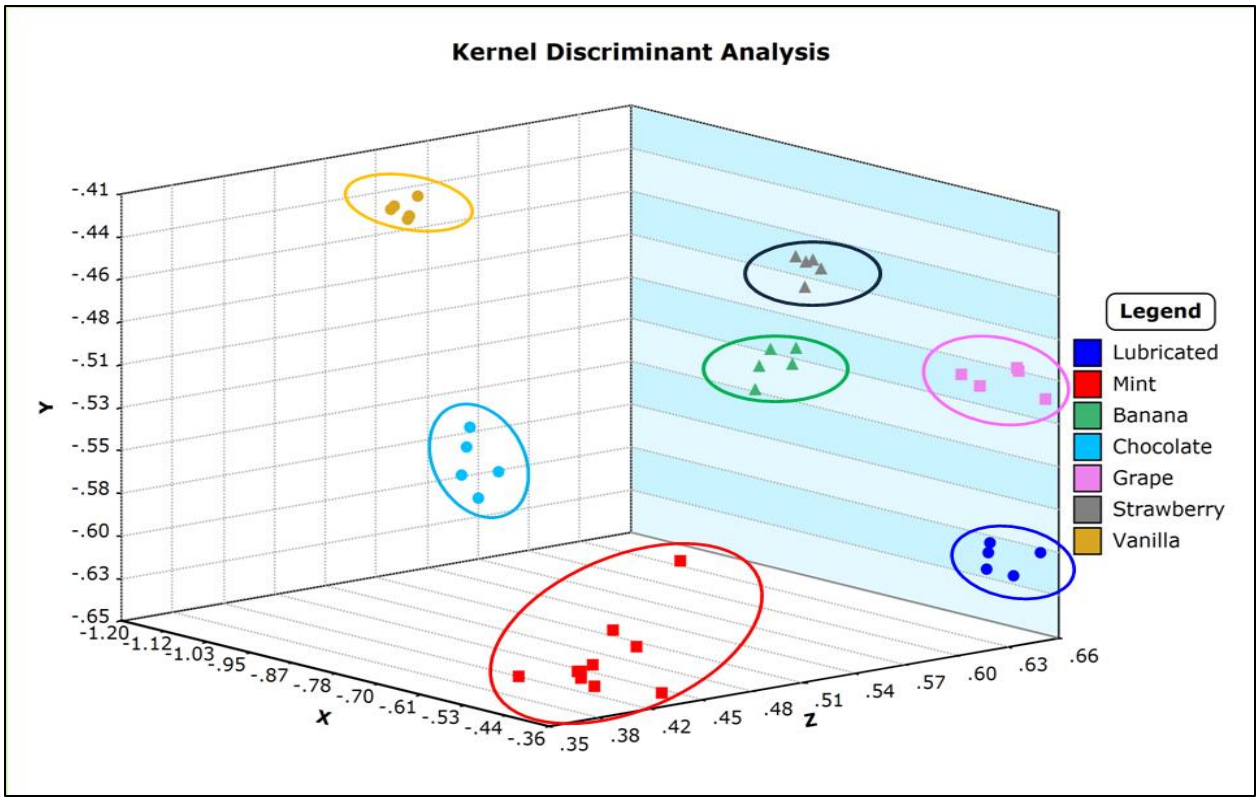

Figure 6. KDA plot for condoms in the brand Fantasy, showing the discrimination between the types of condoms within a single brand. Each symbol in the plot represents a spectra replicate.

differentiated. The circles around the clusters are shown to highlight clustering regions only. The leave-one-out-cross validation (LOOCV) was 90.0% to 100% depending upon the brand.

The raw spectral data for all 110 condom types were also subjected to hierarchical clustering analysis. The result of this type of analysis is a dendrogram (i.e. tree diagram) which illustrates relationships between groups, with closely related groups clustering together. Several different types of dendrograms were generated. One dendrogram contained the entire set of 716 raw spectra collected for all 16 brands. The results of this analysis are shown in Figure 7. Each branch, or clade, ends with a leaf that represents a single raw spectrum. Each leaf of the

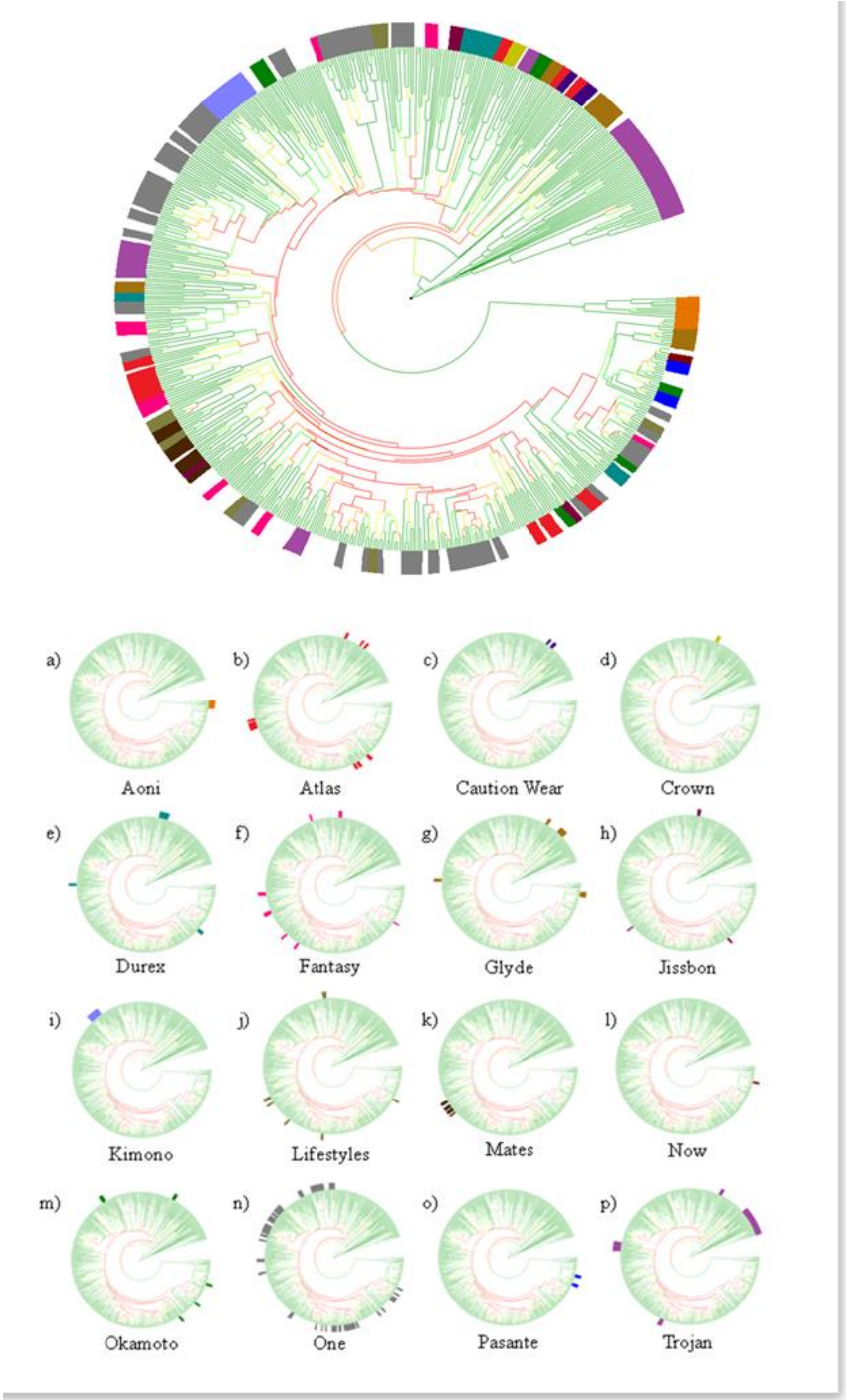

Figure 7. Hierarchical clustering analysis results for the full DART-HRMS dataset that included 716 mass spectra representing 110 condom types within 16 brands. The top image shows the full dendrogram, while the bottom image highlights the area within the dendrogram where the indicated brand falls, with the brand types coded by color.

dendrogram is colored based on the brand of condom. The closer in proximity a leaf or clade is, the more closely related the spectra are. It was therefore expected that condoms of the same brand would cluster with one another. The clustering provides a way in which the relationship between different brands of condoms can be visualized, and a means by which to identify condom residue data based on where it falls in the dendrogram. A second dendrogram was generated from a subset of the spectra. It was comprised of 215 raw spectra of condoms that had distinct lubricant characteristics such as flavoring or warming agents. This represented a total of 37 types from 11 brands. The results are presented in Figure 8. This dendrogram shows that condoms that have distinct lubricant characteristics exhibit high separation between both brands and types. The spectral fingerprint of condoms with specialty chemical characteristics is therefore highly individualistic. It is anticipated that these condoms, with distinct lubricant

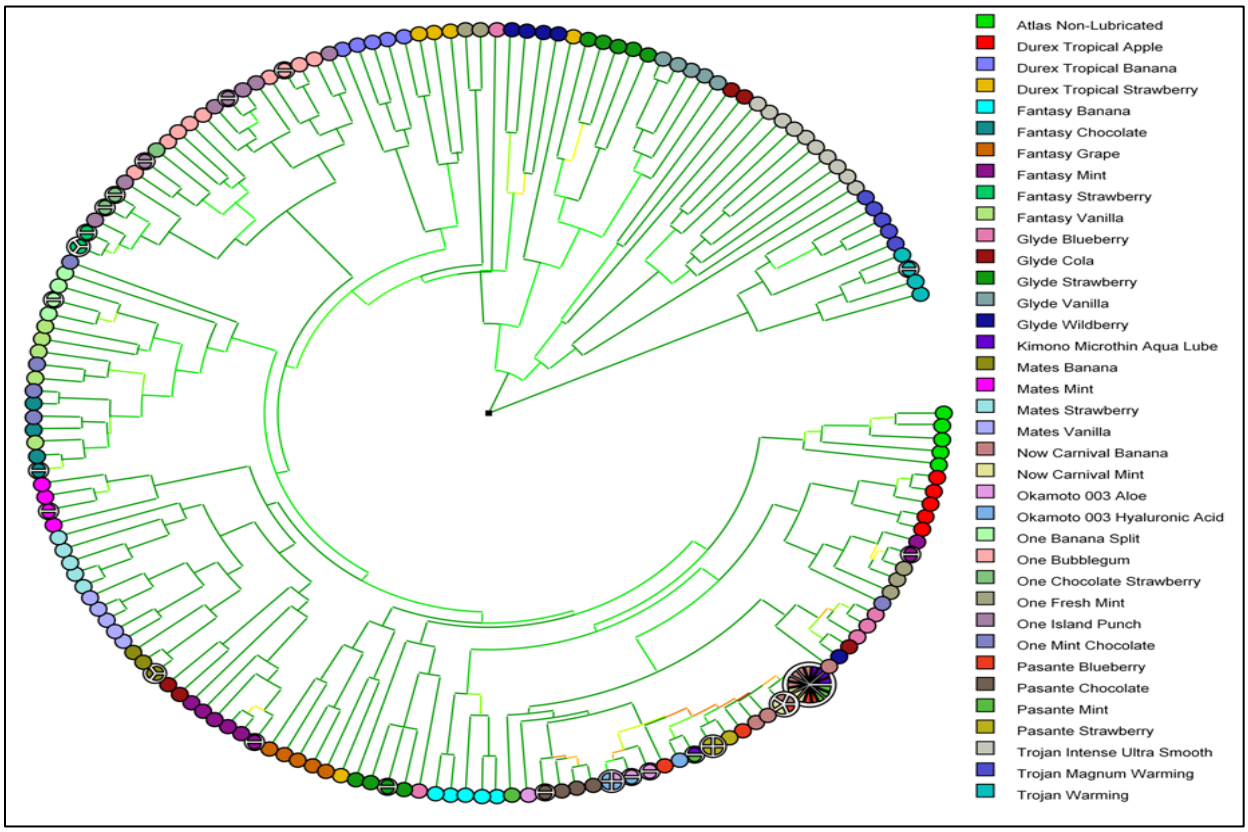

Figure 8. Hierarchical clustering analysis results for the DART-HRMS data set of those condoms with distinct lubricant characteristics that included 215 mass spectra representing 37 condom types within 11 brands. The type of condom is coded by color.

characteristics, would have the highest probability of being correctly classified. The hierarchical clustering results serve as proof-of-principle that condom residues can be identified by an unsupervised statistical analysis method.

One question that arose in the course of this study is whether "universal" condom markers exist. Such compounds would be those whose presence provides strong evidence or "proof" that a trace material is condom-lubricant derived. To be useful, such compounds would need to be present in most, if not all, condoms. If such molecules exist, it would be beneficial to have this information because then, a single analysis approach could be developed that would allow assessment of whether trace evidence found by crime scene investigators is from a condom. Careful visual inspection of the spectra acquired in this study revealed that there were several compounds that were present in most condoms, but absent in only a few. These included *N*,*N*-dibutylformamide, isonox 132 (2, 6-di-*tert*-butyl-4-*sec*-butylphenol), oleamide, and acetone anil. This observation was an important factor in enabling successful classification of condoms by statistical analysis. A summary of this finding is shown in a Venn diagram in Figure 9. Each of the four universal compounds, *N*,*N*-dibutylformamide, Isonox 132, oleamide, and acetone anil is represented by different colors (yellow, green, blue, and green for *N*,*N*-dibutylformamide, Isonox 132, acetone anil and oleamide respectively). Entries in non-overlapping regions indicate that the noted condom type did not contain the indicated compound. For example, Okamoto Ultra Smooth did not contain *N*,*N*- dibutylformamide, but did contain Isonox 132, oleamide, and acetone anil. Entries in the overlapping regions indicate condoms that did not contain the compounds represented. For example, Now Super Fine did not contain oleamide and acetone anil. The combined use of all four of these "universal" compounds could enable rapid

determination of whether a residue is condom-derived for any of the condoms analyzed in this study.

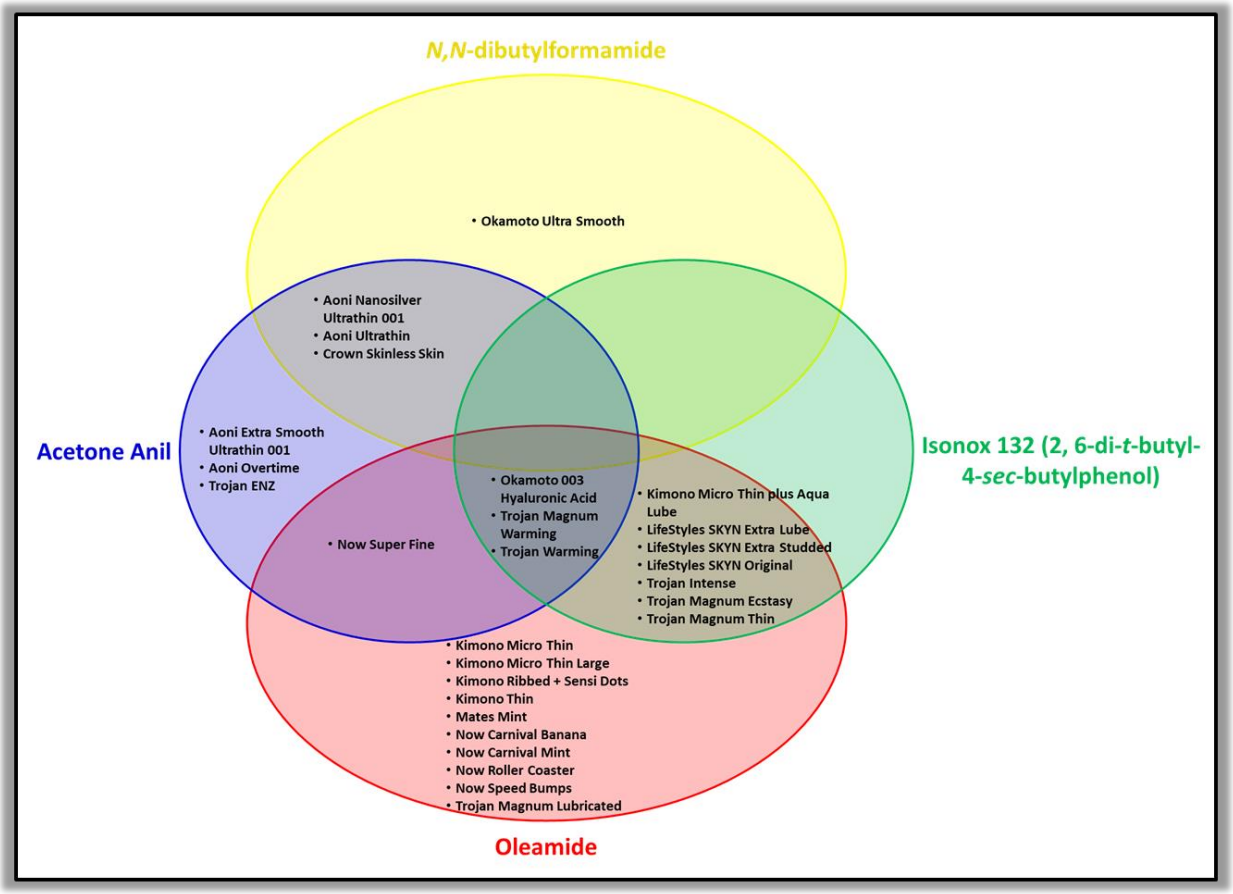

Figure 9. Venn diagram showing a subset of four compounds detected to be present in most of the condoms anaylzed. *N*,*N*-dibutylformamide, Isonox 132, acetone anil, and oleamide are represented in yellow, green, blue, and red respectively. The named condom types are those in which the indicated compounds are absent.

Conclusions

The analysis of condom lubricants representing 110 types from 16 brands manufactured in different parts of the world by DART-HRMS shows that each condom exhibits a diagnostic chemical fingerprint. Each spectrum was acquired within a few seconds, and no sample preparation was necessary, as the material could be analyzed in its native form. Chemical fingerprints were found to be consistent for different batches of the same type of condom, and

these signatures were different from those of other condom types. DART-HRMS also revealed within the spectra the presence of masses that were consistent with both compounds commonly observed in condoms, and those that would be expected to be present based on advertised attributes, such as flavoring agents. Nevertheless, even for condoms advertised to have the same given characteristic (such as a flavor), the chemical signatures for different types remained distinct.

The rapidity with which spectral replicates could be acquired made possible the compilation of >700 spectra which could be subjected to multivariate statistical analysis processing. Kernel discriminant analysis revealed that condom types within a given brand could be readily distinguished and identified with 90-100% accuracy, simply from screening the condom residue against the group of compiled spectra. Furthermore, hierarchical clustering analysis showed that condom types within a brand clustered together (enabling their identification), and that condoms with specialty features could also be differentiated. Among the reasons for the ability to distinguish between and identify the condom lubricants was the finding that several compounds commonly found in condoms were absent from a particular subset of them. These molecules included acetone anil, *N*,*N*-dibutylformamide, Isonox 132 and oleamide. The finding that these compounds are present in most condoms makes them primary markers that can be used to identify trace evidence which contains any one or a combination of them as condom-derived residue.

The observations and findings of this study may be of practical utility in assisting crime scene investigators and medical science practitioners in identifying condom-derived trace evidence. The developed database may be used as a tool against which condom lubricant unknowns can be screened in order to determine the condom type and its corresponding brand.

The identification of a subset of primary condom markers paves the way for future studies aimed at investigating their lifetimes in biological fluids, which will be important in analysis of rape test kits. Future studies should explore the extent to which DART-HRMS analysis is compatible with direct analysis of rape test kit swabs.

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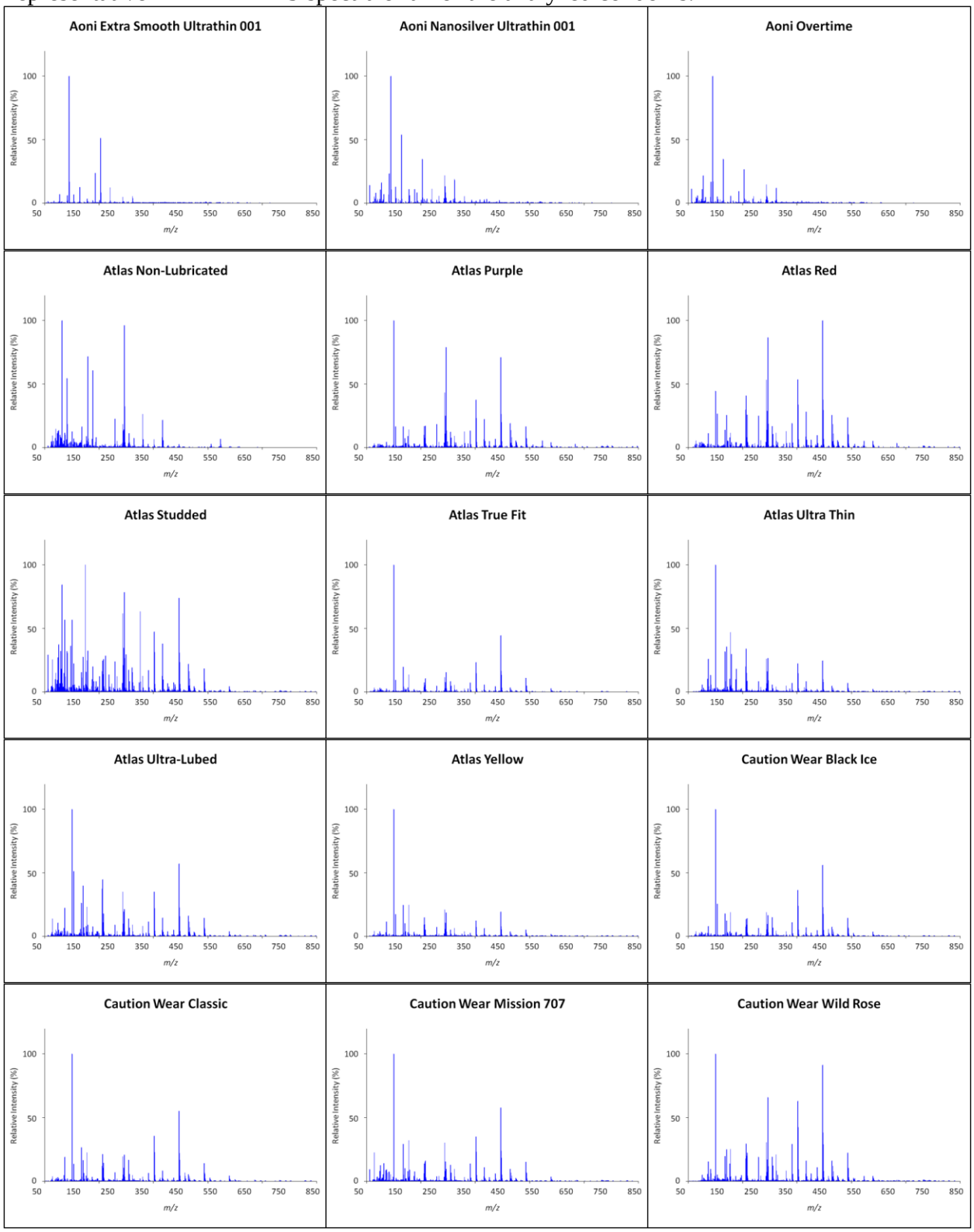
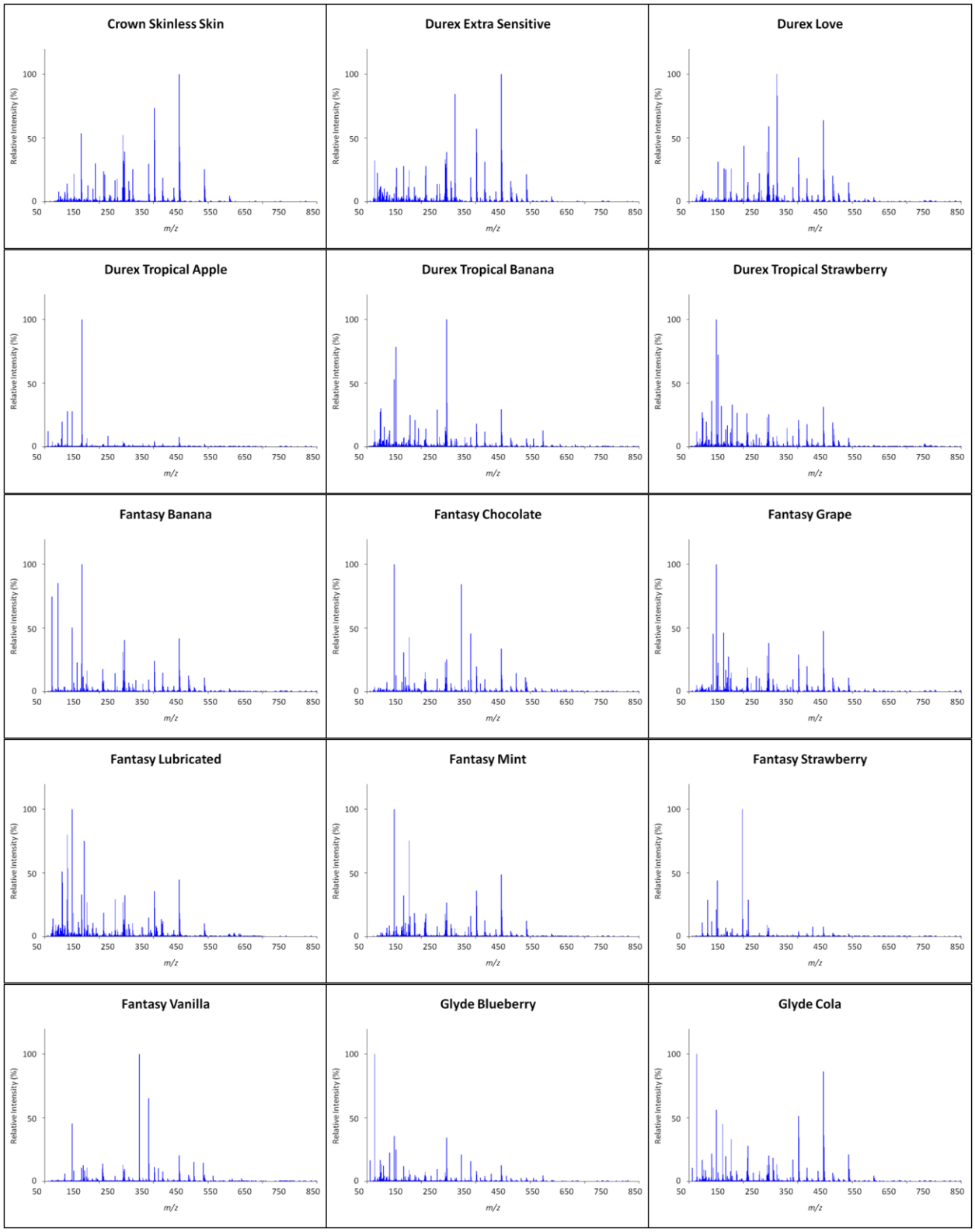
Appendix

Representative DART-HRMS spectra of all of the analyzed condoms.




Crown Skinless Skin
Durex Extra Sensitive
Durex Love
Durex Tropical Apple
Durex Tropical Banana
Durex Tropical Strawberry
Fantasy Banana
Fantasy Chocolate
Fantasy Grape
Fantasy Lubricated
Fantasy Mint
Fantasy Strawberry
Fantasy Vanilla
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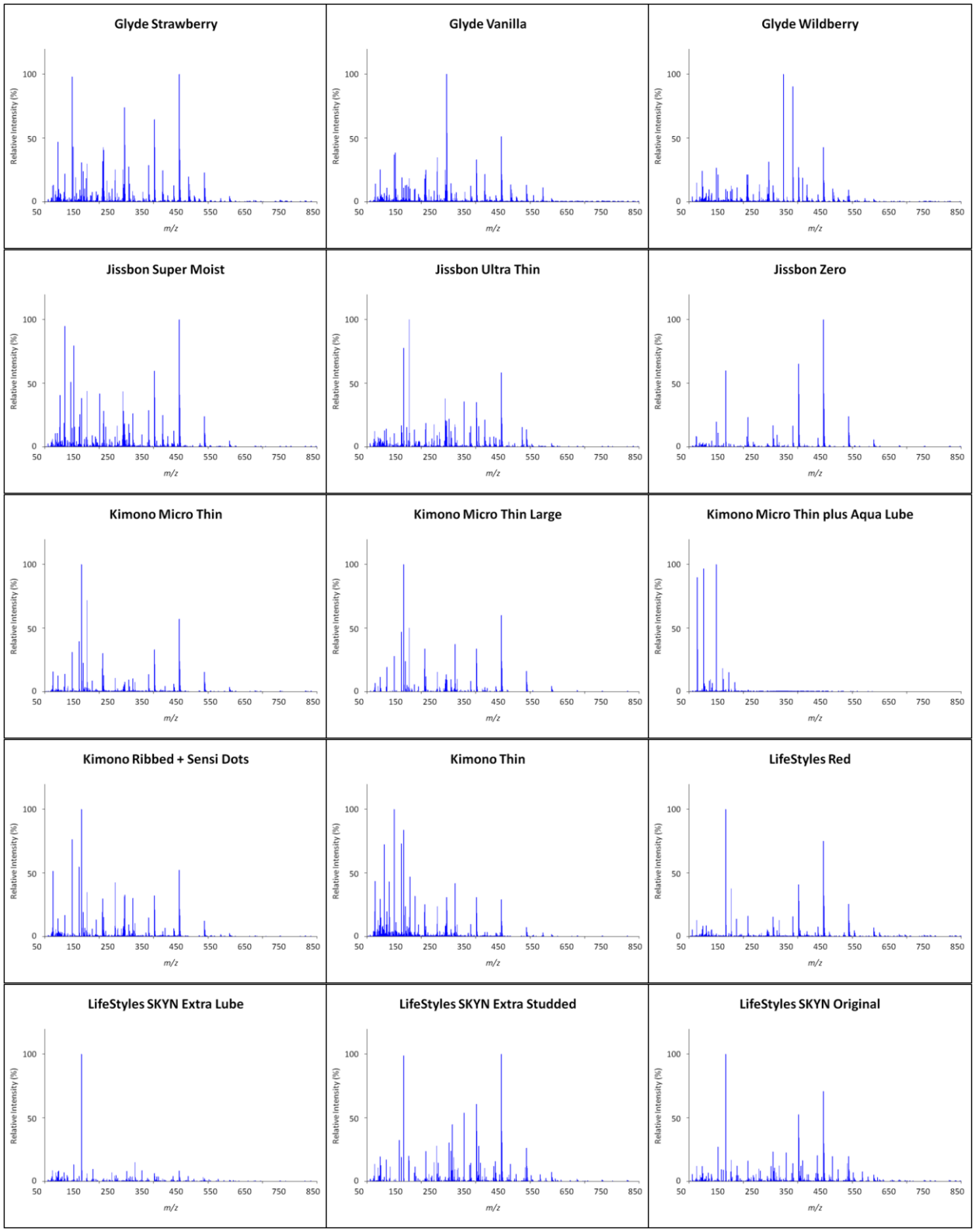
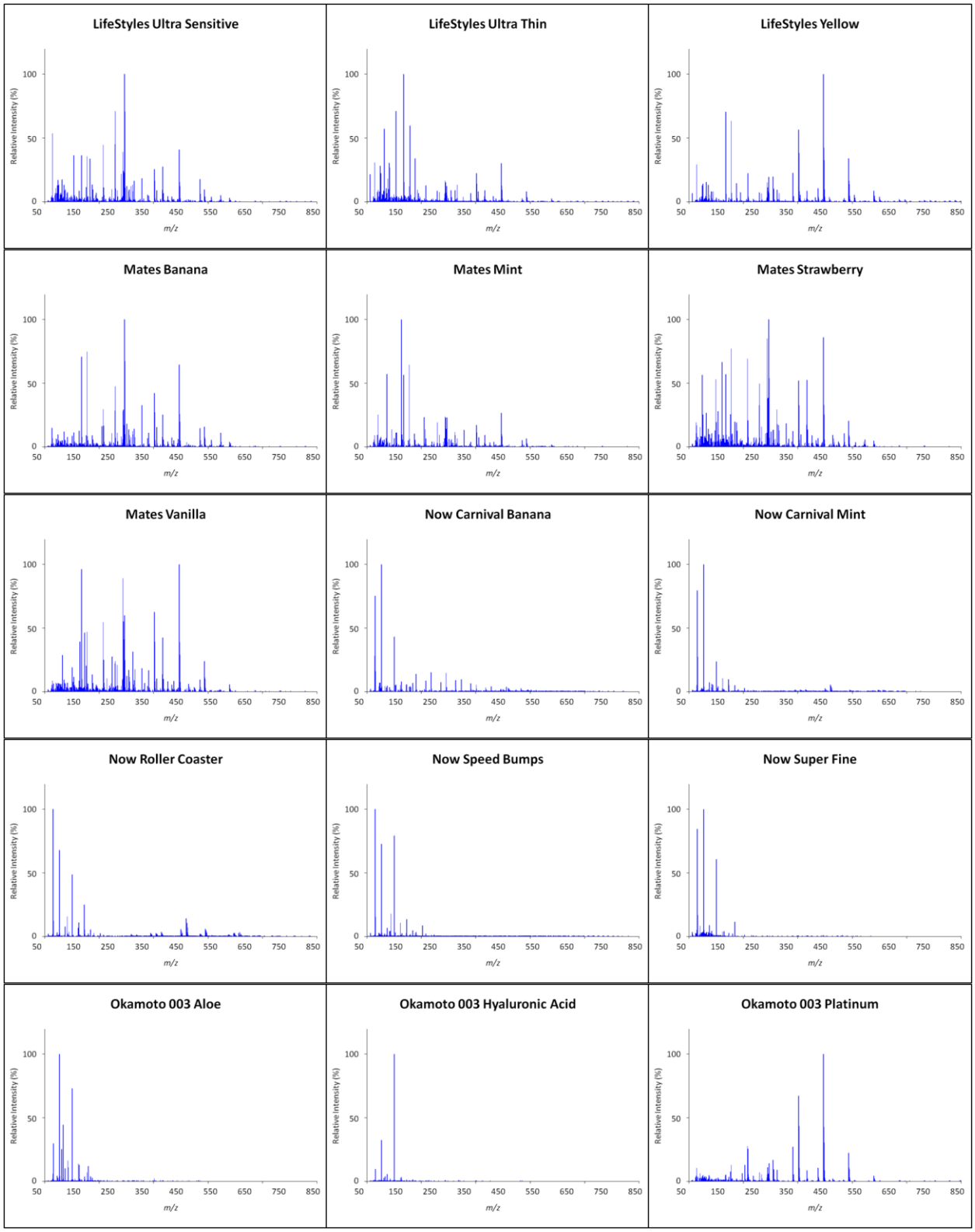
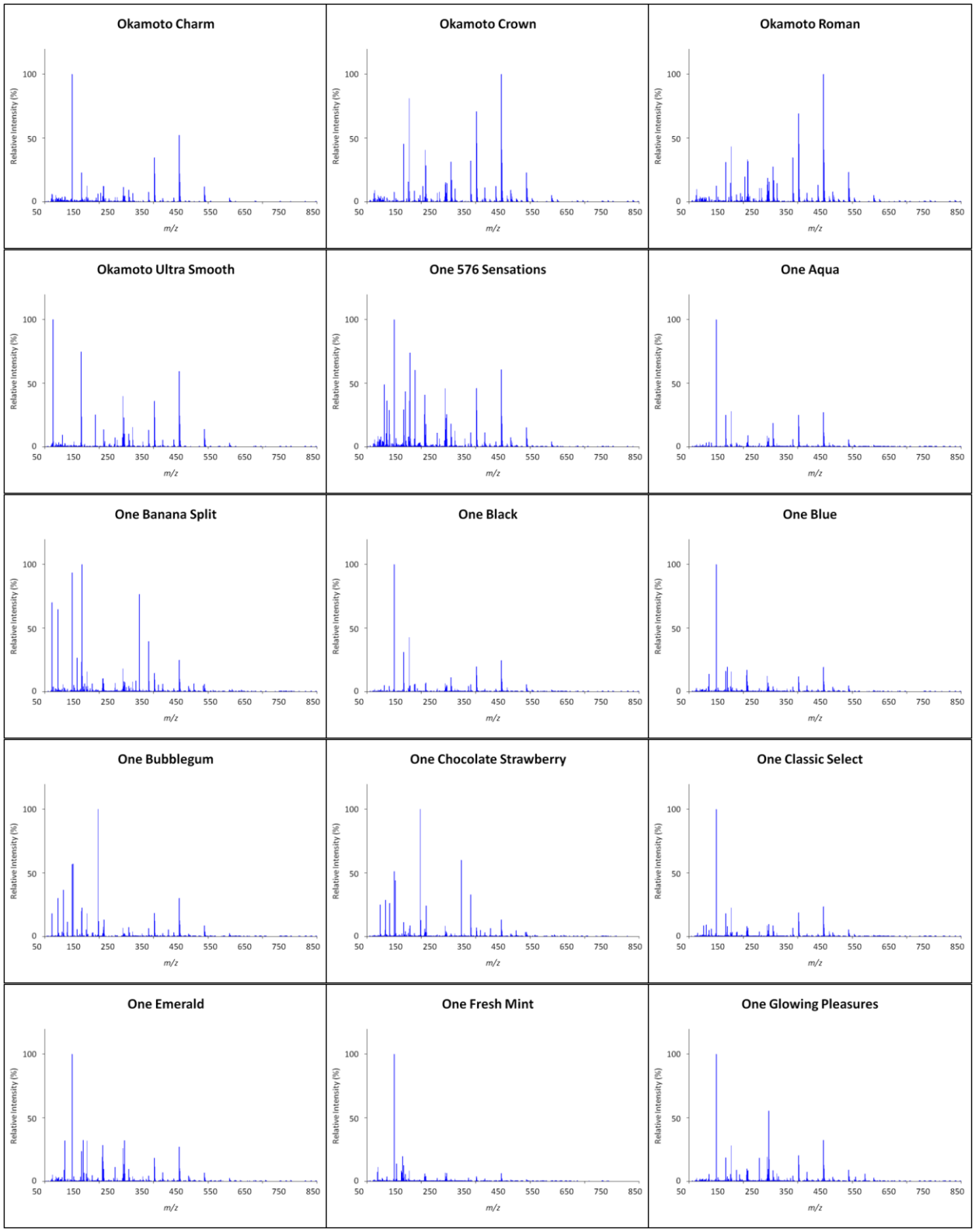
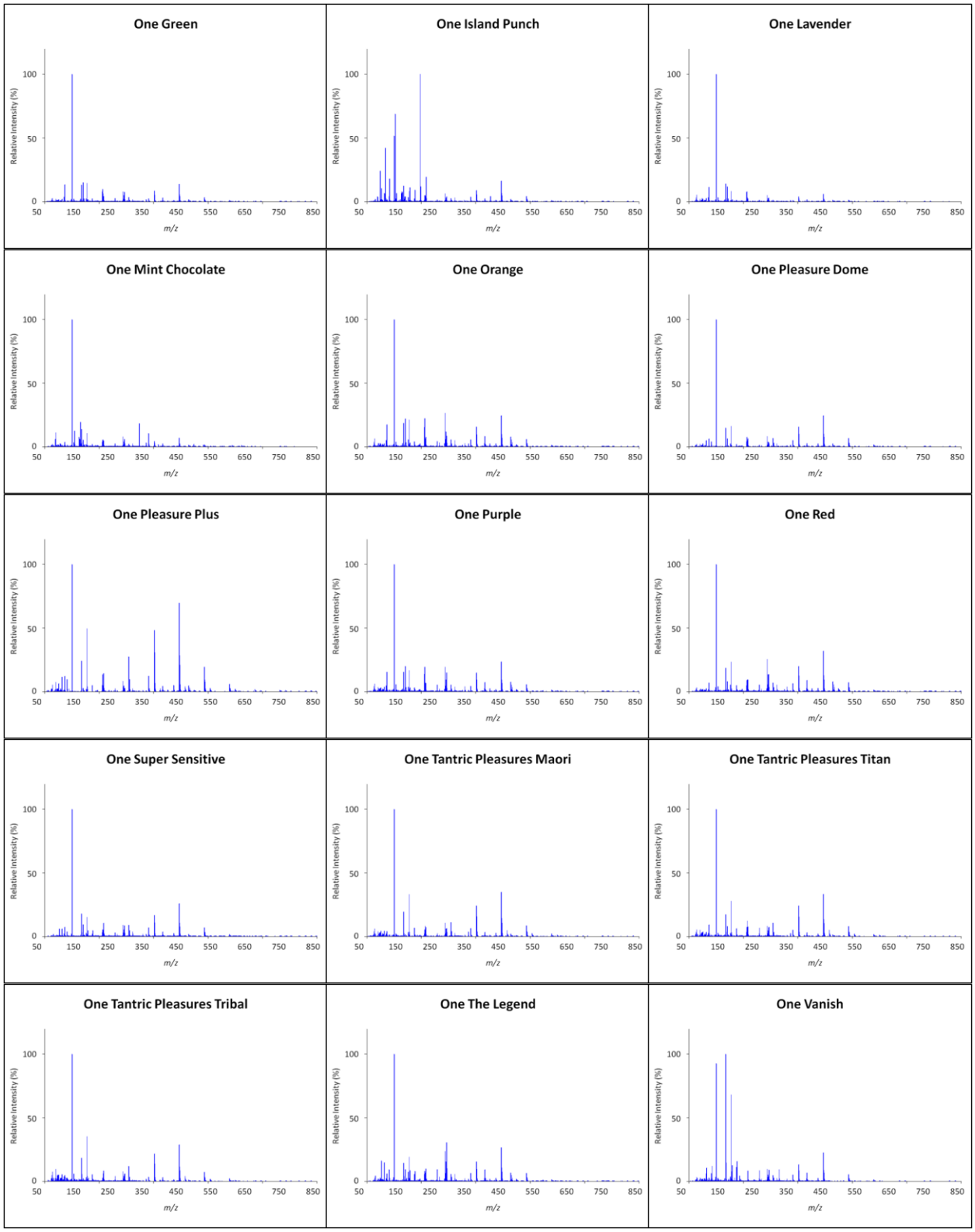
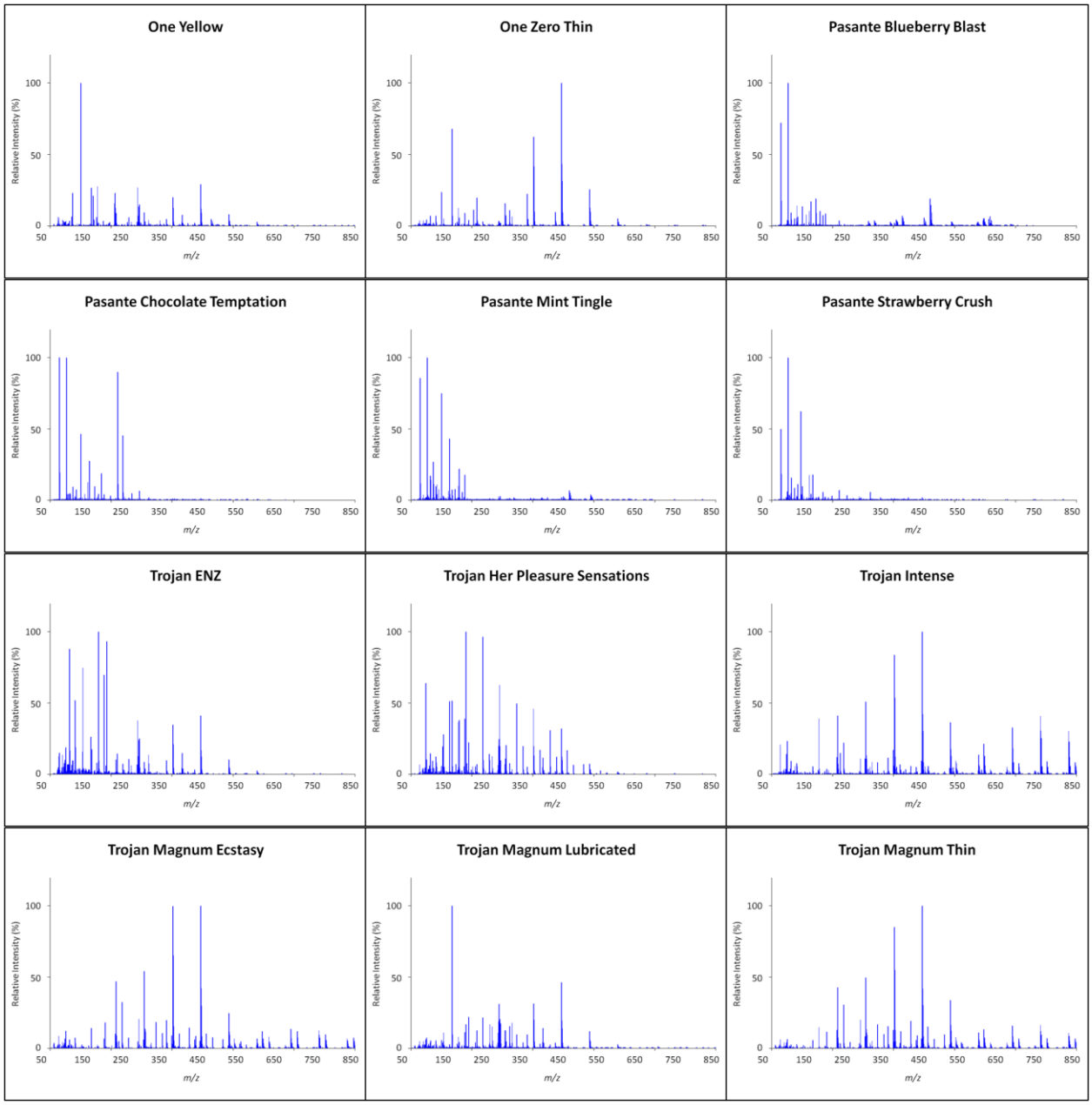
100
50
150
250
350
450
550
650
750
850

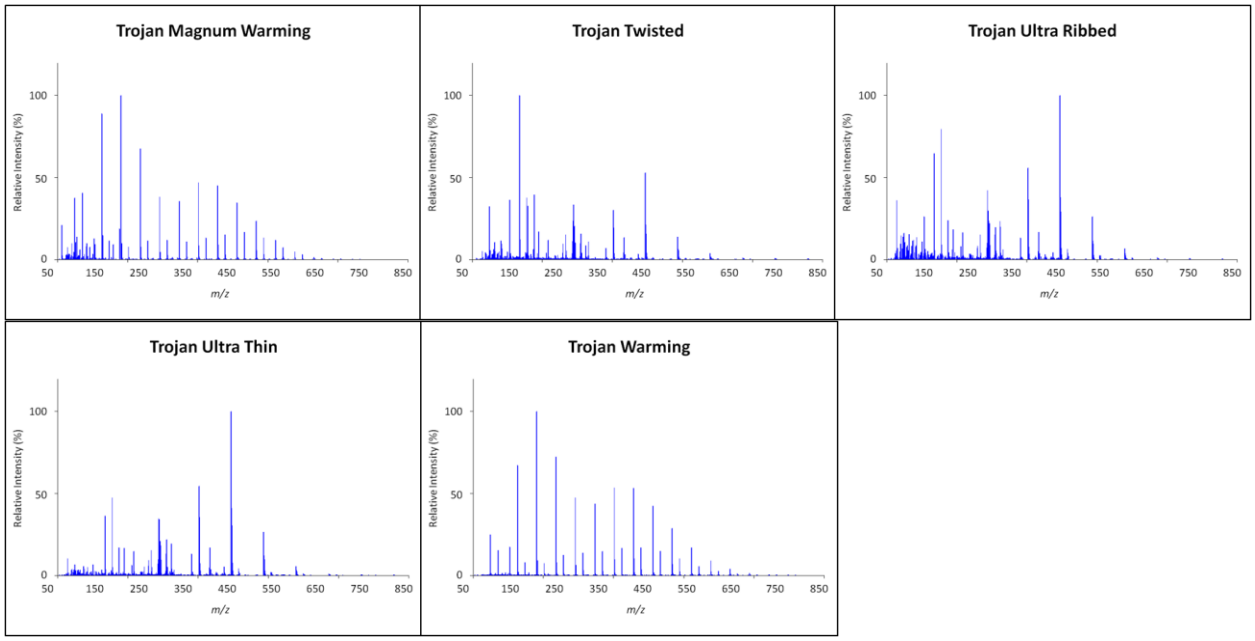

Glyde Strawberry
Glyde Vanilla
Glyde Wildberry
Jissbon Super Moist
Jissbon Ultra Thin
Jissbon Zero
Kimono Micro Thin
Kimono Micro Thin Large
Kimono Micro Thin plus Aqua Lube
Kimono Ribbed + Sensi Dots
Kimono Thin
LifeStyles Red
LifeStyles SKYN Extra Lube
LifeStyles SKYN Extra Studded
LifeStyles SKYN Original
Relative Intensity (%)
m/z
0
50
100
50
150
250
350
450
550
650
750
850


LifeStyles Ultra Sensitive
LifeStyles Ultra Thin
LifeStyles Yellow
Mates Banana
Mates Mint
Mates Strawberry
Mates Vanilla
Now Carnival Banana
Now Carnival Mint
Now Roller Coaster
Now Speed Bumps
Now Super Fine
Okamoto 003 Aloe
Okamoto 003 Hyaluronic Acid
Okamoto 003 Platinum
Relative Intensity (%)
m/z


Okamoto Charm
Okamoto Crown
Okamoto Roman
Okamoto Ultra Smooth
One 576 Sensations
One Aqua
One Banana Split
One Black
One Blue
One Bubblegum
One Chocolate Strawberry
One Classic Select
One Emerald
One Fresh Mint
One Glowing Pleasures
Relative Intensity (%)
m/z


One Green
One Island Punch
One Lavender
One Mint Chocolate
One Orange
One Pleasure Dome
One Pleasure Plus
One Purple
One Red
One Super Sensitive
One Tantric Pleasures Maori
One Tantric Pleasures Titan
One Tantric Pleasures Tribal
One The Legend
One Vanish
Relative Intensity (%)
m/z
0
50
100
50
150
250
350
450
550
650
750
850


One Yellow
One Zero Thin
Pasante Blueberry Blast
Pasante Chocolate Temptation
Pasante Mint Tingle
Pasante Strawberry Crush
Trojan ENZ
Trojan Her Pleasure Sensations
Trojan Intense
Trojan Magnum Ecstasy
Trojan Magnum Lubricated
Trojan Magnum Thin
Relative Intensity (%)
m/z
0
50
100
50
150
250
350
450
550
650
750
850


Trojan Magnum Warming
Trojan Twisted
Trojan Ultra Ribbed
Trojan Ultra Thin
Trojan Warming
Relative Intensity (%)
m/z
0
50
100
50
150
250
350
450
550
650
750
850