Application Note

EMPOWERING RESULTS
Elemental Analysis | GC Mass Spectrometry | Metallography

Instrument: Pegasus® BTX and Pegasus HRT+

Characterization of Extractables from Dental Bite Guards Using GC-TOFMS

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Key Words: Extractable, Leachable, E&L, Medical Devices, ISO 10993, GC, MS, TOFMS, HR-TOFMS, Non-Target Analysis, Deconvolution, RI, Retention Index

Introduction

The characterization of medical devices for their potential extractable and leachable profile is important. These evaluations are part of the risk management process for medical devices, outlined in ISO 10993 Part 18, that helps to determine which analytes could leach from a medical device so that the associated toxicological risks of exposure to those analytes can be understood. Chemical evaluation of the extracted or leached samples is a crucial part of these procedures because it is necessary to first determine and identify the chemicals in order to know what risks they may pose. These investigations are generally non-targeted and can be challenged by sample complexity and the detection requirements that are often low-level.

Gas chromatography with time-of-flight mass spectrometry (GC-TOFMS) is well suited to address these challenges. Individual analytes are chromatographically isolated with GC, and TOFMS detection provides information to support identifications. The *Pegasus* BTX TOFMS system offers non-targeted detection with exceptional sensitivity for the characterization of both volatile and semi-volatile chemical constituents in these types of samples. Acquiring full m/z range data allows for spectral searching against library databases, and the non-skewed spectral data is optimal for mathematical deconvolution algorithms that enhance the separation power of the system. The *Pegasus* HRT+ GC-TOFMS system adds high resolution, accurate mass spectral data. When needed, this provides additional information to characterize unknown extractables that may not be present in commercial MS databases.

In this work, extracts for a variety of dental bite guards were analyzed as a case study to demonstrate aspects of the ISO 10993 risk management analytical workflow. These types of dental bite guards are characterized as medical devices that require this screening. They can be prescribed by dentists to prevent tooth grinding during sleep and can also be purchased over the counter. Here, extracts for over-the-counter dental bite guards were prepared and analyzed by GC-TOFMS. The analytical evaluation threshold (AET) was determined and analytes above this threshold were investigated. The *Pegasus* BTX was used for the initial screen of all samples and the *Pegasus* HRT+ was used when samples had analytes above the AET without a library identification. The mass spectral data and chromatographic elution information were leveraged to better characterize the chemical composition of these extracts.

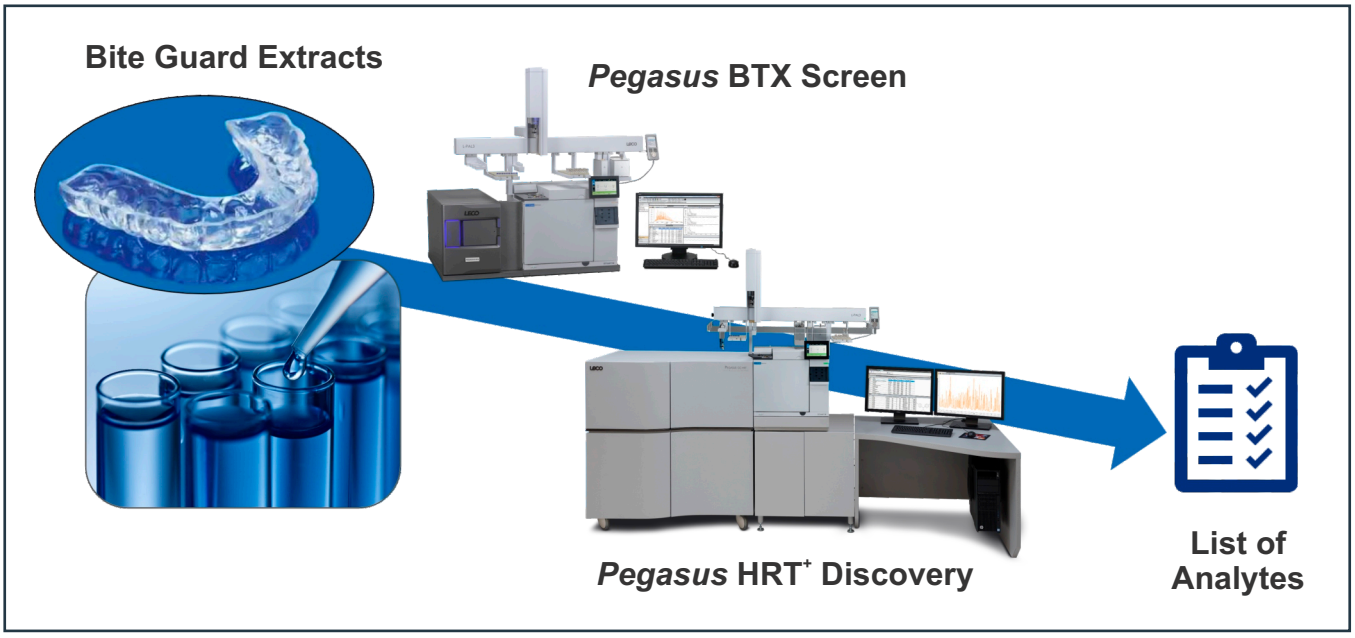


Figure 1. The Pegasus BTX and Pegasus HRT+ provided detailed analyte information for extracts of dental bite guard samples.

Experimental

Commercially available over-the-counter dental bite guards made of various materials, including silicone and ethyl vinyl acetate, were purchased for analysis. Extracts for each bite guard were prepared with guidance from ISO 10993 for an exaggerated extraction. Two solvent extractions, hexane and isopropyl alcohol, were performed for each dental bite guard. For each, 1 g of the sample was submerged in 5 mL of solvent. The vial was then heated at 50 °C for 72 hours with agitation. The bite guards completely dissolved in the hexane, so only the isopropyl alcohol extracts were further evaluated. The samples were centrifuged for 20 min at 4K rpm and the supernatant was collected for analysis. All isopropyl alcohol extracts were analyzed with the *Pegasus* BTX as described in Table 1. An alkane standard was analyzed for retention index (RI) determinations and a subset of the FDA CLAP extractables standard was analyzed at 10 ppm for determination of the AET. Extracts with unknown analytes above the AET were also analyzed with the Pegasus HRT as described in Table 2. Standards for the proposed structures of these unknowns were purchased and also analyzed to confirm the identifications.

Table 1. *Pegasus* BTX GC-TOFMS Method Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	1 µL, split 10:1
Gas Chromatograph	
Inlet	310 °C
Carrier Gas	He @ 1 mL/min
Columns	Rxi-5HT, 30 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	Hold 1 min at 40 °C, ramp 7.5 °C/min to 340 °C (hold 10 min)
Transfer Line	325 °C
Mass Spectrometer	**LECO *Pegasus* BTX**
Ion Source Temperature	250 °C
Mass Range	35-650 m/z
Acquisition Rate	10 spectra/s

Table 2. *Pegasus* HRT$^+$ TOFMS Method Conditions

Mass Spectrometer	**LECO *Pegasus* HRT$^+$**
LECO Source	LECO MMS (EI and CI, methane)
Ion Source Temperature	250 °C (EI) and 165 °C (PCI)
Mass Range	35-750 m/z (EI) and 60-800 (CI)
Acquisition Rate	10 spectra/s

Results and Discussion

Representative chromatograms for all of the bite guard extracts, analyzed with the *Pegasus* BTX, are shown in Figure 2. Most of the sample chromatograms are dominated by a very intense peak that elutes after 1000 s in all of the sample extracts. Many other less intense analytes are also present in these samples.

LECO's ChromaTOF® software non-target peak finding can be applied to all of the samples to determine the analytes present in these extracts, including these intense peaks and also the analytes with less intensity that are harder to see in these chromatograms. Peak finding provides deconvoluted spectra that are searched against spectral databases for tentative identifications. Experimental RI values based on the alkane standard are also calculated with data processing. Comparing the calculated RI to library RI further supports the identifications. For example, peak finding results reveal that the major peak eluting shortly after 1000 s is butylated hydroxytoluene (BHT), as shown in Figure 3. This identification is based on spectral matching with a similarity score of 924 of a possible 1000 and is also supported with RI as the observed RI of 1516 compares well to the library RI value of 1513. BHT is an antioxidant and preservative often used in these types of materials.

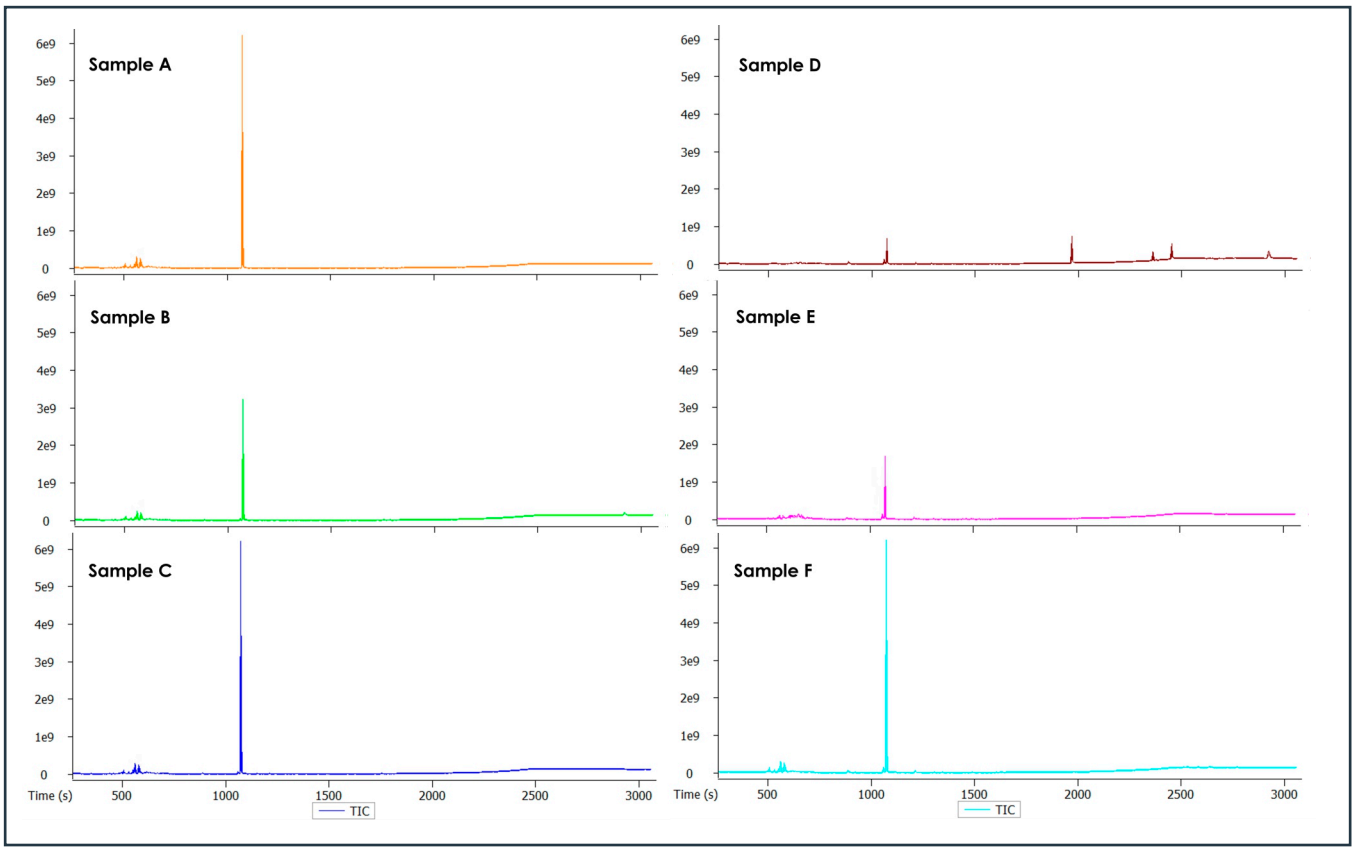
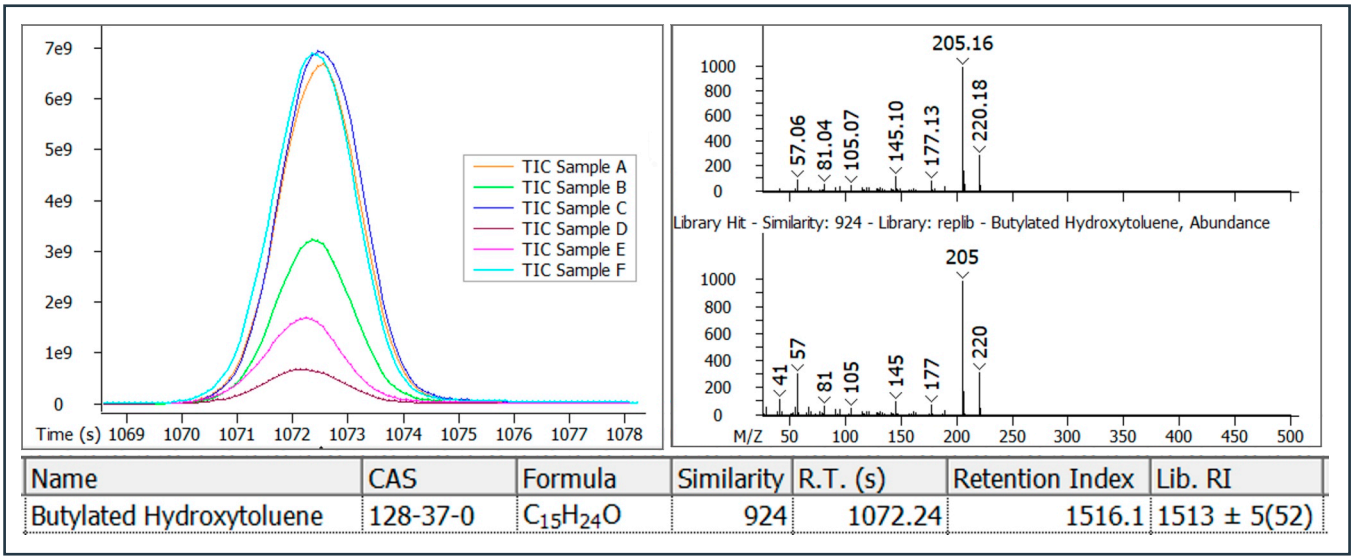


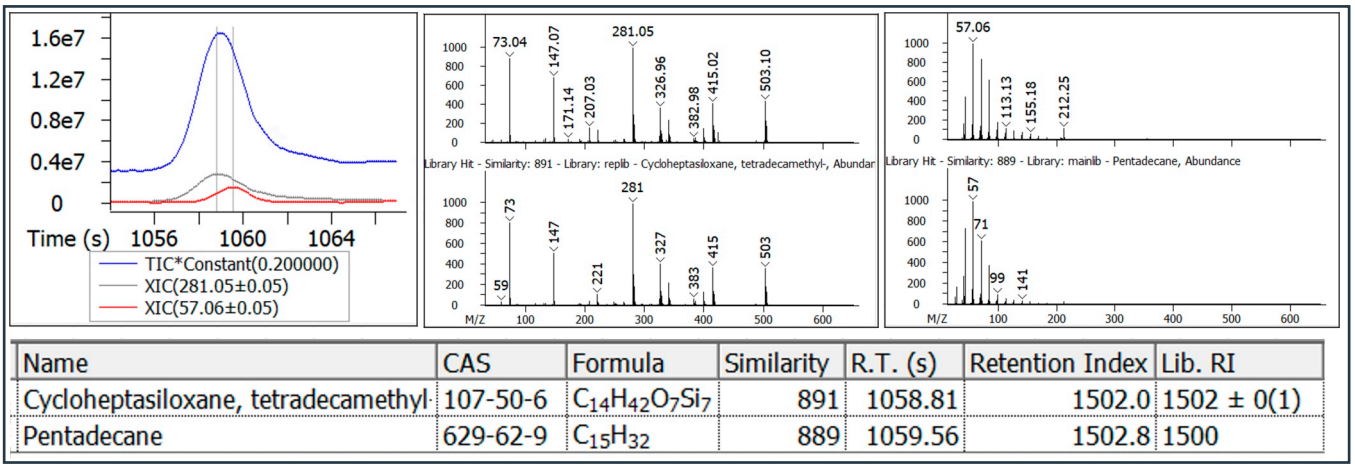
Figure 2. Pegasus BTX TIC chromatograms of representative extracts for each of the dental bite guards are shown.



Name	CAS	Formula	Similarity	R.T. (s)	Retention Index	Lib. RI
Butylated Hydroxytoluene	128-37-0	$C_{15}H_{24}O$	924	1072.24	1516.1	1513 ± 5(52)

Figure 3. The antioxidant BHT was determined in all of the extract samples. Spectrum and peak finding results for sample E are shown.

Other features are also determined with *ChromaTOF* non-target peak finding, even if they are present at lower intensity and/or have chromatographic coelution. For example, a siloxane peak and pentadecane are shown in Figure 4. These identifications are also supported with spectral and RI matching, as indicated.



Name	CAS	Formula	Similarity	R.T. (s)	Retention Index	Lib. RI
Cycloheptasiloxane, tetradecamethyl	107-50-6	$C_{14}H_{42}O_7Si_7$	891	1058.81	1502.0	1502 ± 0(1)
Pentadecane	629-62-9	$C_{15}H_{32}$	889	1059.56	1502.8	1500

Figure 4. Deconvolution mathematically separates chromatographic coelutions. A siloxane and pentadecane coelute in the extract for Sample C.

While peak finding can efficiently uncover this information for compounds across the peak intensity range, one of the questions in screening the medical devices with ISO 10993 is which of the determined analyte peaks are present at levels of potential concern and need to be reported for further toxicological evaluation. One consideration is if the observed analyte came from the sample itself or the sampling procedure and this can be checked by comparing the extract to a method blank. For example, the siloxane peak shown in Figure 4 is also observed in a method blank (not shown) so it is not determined to be an analyte of potential concern. Another consideration is based on the concentration or peak intensity, as this relates to the level of potential exposure. Neither BHT or pentadecane, shown in Figures 3 and 4, were observed in the blank so they may need to be reported for further evaluation. As shown, though, these analytes are present at very different peak intensities. BHT has a higher S/N and pentadecane is observed at a lower S/N. Part of the workflow is to determine the intensity threshold cutoff and the AET, described in ISO Method 10993, is used for this purpose.

The AET is calculated as described in Formula 1 where DBT is the dose-based threshold, A is the number of devices used for the extract, B is the volume of the extract, C is the clinical exposure in number of devices per day, and UF is the uncertainty factor.

$$\text{Formula 1} \quad AET = \frac{DBT \times (A/BC)}{UF}$$

This calculation provides a concentration threshold for reporting, which can be connected with chromatographic intensity by analyzing a standard at this concentration. Features with an intensity below this level are considered to have exposures below the threshold of potential concern and those above the level are to be reported for additional toxicological investigation. For these samples and extracts, the calculated AET was 10 ppm. As such, a subset of the FDA CLAP extractables standards were analyzed at 10 ppm as shown in Figure 5. The average peak height of these standards was 0.62e8, indicated with the horizontal line in Figure 5, which was used as the intensity threshold cutoff for the AET.

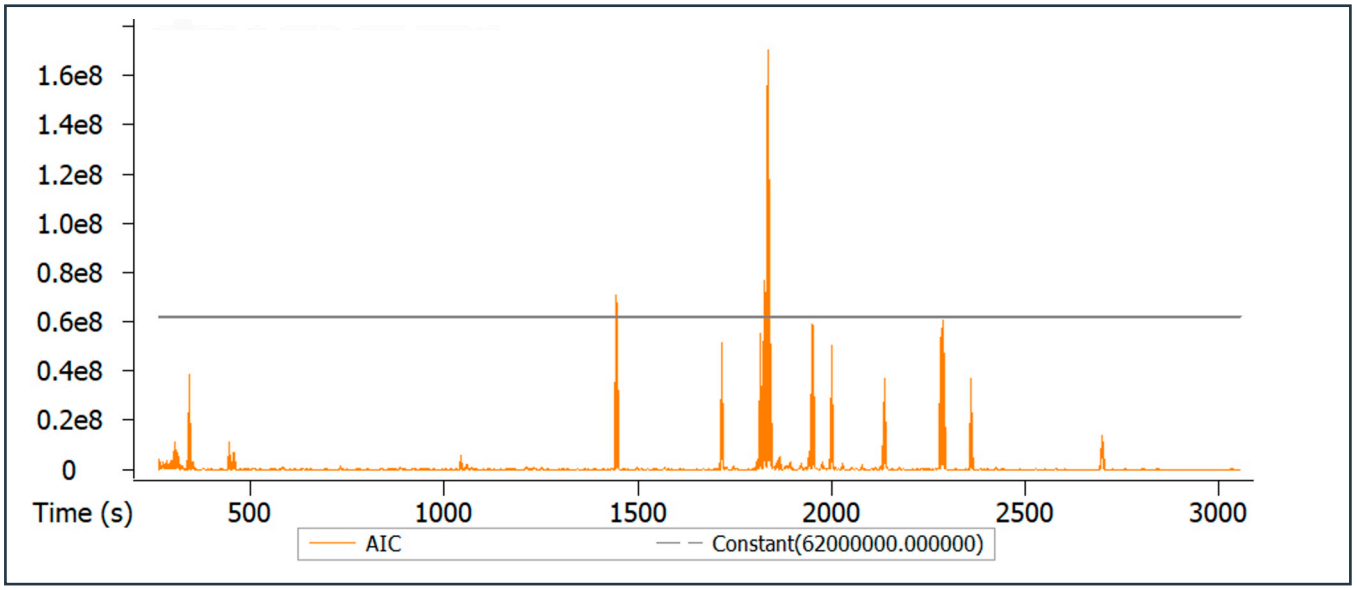


Figure 5. A portion of the FDA CLAP Standards were analyzed at 10 ppm to determine the AET intensity threshold.

Samples A-F are shown again in Figure 6 with the AET threshold indicated. The y-axes for these plots are zoomed compared to the chromatograms shown in Figure 2. BHT, shown in Figure 3, exceeded the AET in all of the samples. The siloxane peak, shown in Figure 4, exceeds the AET in samples B-F, but was present in the blank, as previously noted. Pentadecane, shown in Figure 4, did not exceed the AET in any of the samples. Samples A, B, C, E, and F have some early eluting linear and branched alkanes that also exceed the AET. And, Sample D has 4 additional later eluting peaks above the AET. The latest eluting peak in Sample D is also observed in Sample B.

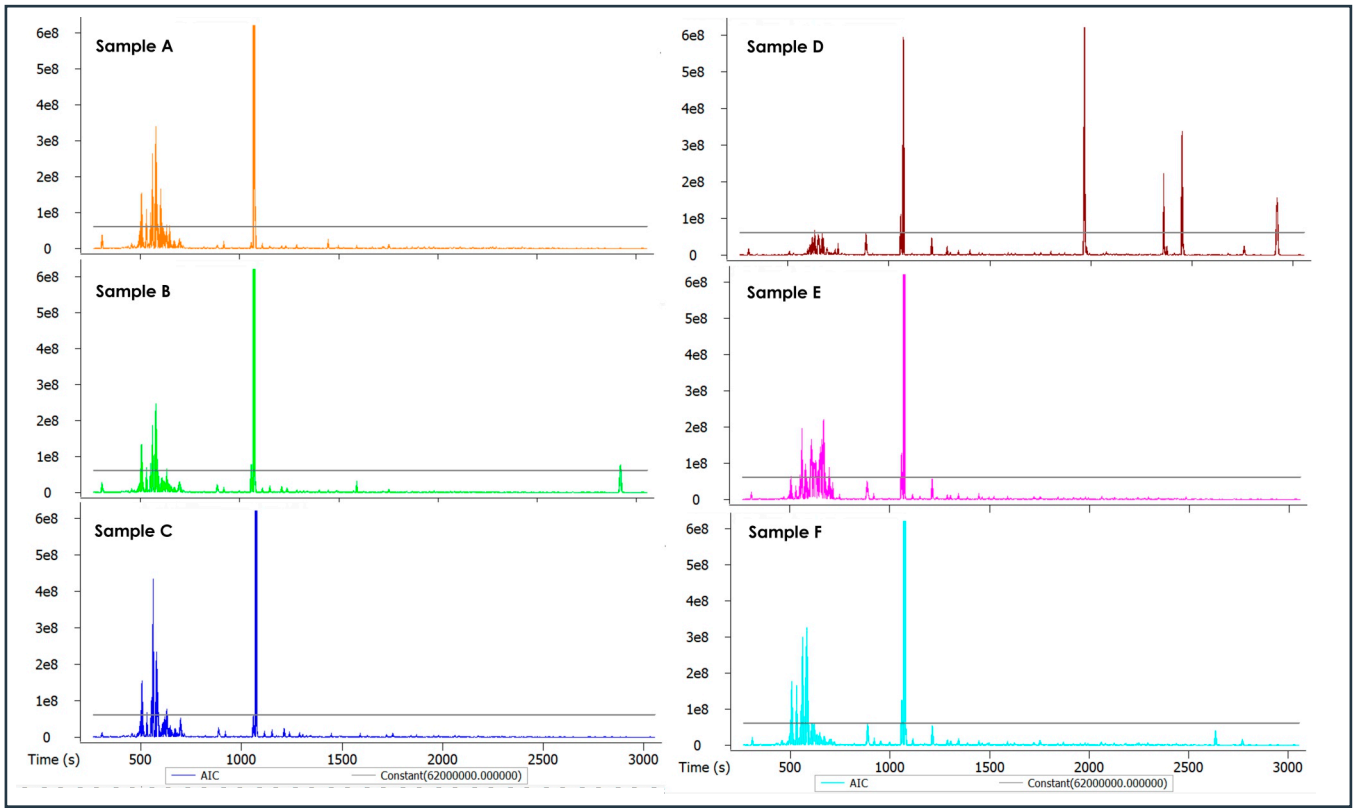


Figure 6. The extracts for all 6 samples are shown with the AET threshold indicated. The chromatograms have been zoomed on the y-axis compared to Figure 2.

The 5 peaks exceeding the AET in Sample D are described in Table 3. Three of the five analytes were identified based on spectral matching to libraries and RI agreement with library database information. BHT is one of the five, as previously discussed. Erucamide, a fatty acid slip agent, and Irganox 1076, an antioxidant, were also determined with spectral and RI matching. The other two analytes were unknowns.

Table 3. Peak find information for the analytes exceeding the AET in Sample D

Name	CAS	Formula	Similarity	R.T. (s)	Retention Index	Lib. RI
Butylated Hydroxytoluene	128-37-0	$C_{15}H_{24}O$	912	1072.18	1516.1	1513 ± 5(52)
Erucamide	112-84-5	$C_{22}H_{43}NO$	858	1967.46	2782.8	2793 ± 0(1)
Irganox 1076	2082-79-3	$C_{35}H_{62}O_3$	863	2360.46	3620.8	3603 ± 0(1)
Peak 1395				2451.2	3846.4	
Peak 1449				2921.58	5024.6	

Spectral information for both of the unknowns is shown in Figure 7. The raw acquired spectra and the deconvoluted spectra are both shown. Deconvolution can address coelutions, as shown in Figure 4, and it can also help remove background to provide cleaner spectra for interpretation, as shown here. However, even with deconvoluted spectra, these analytes were not able to be identified by searching commercially available mass spectral library databases.

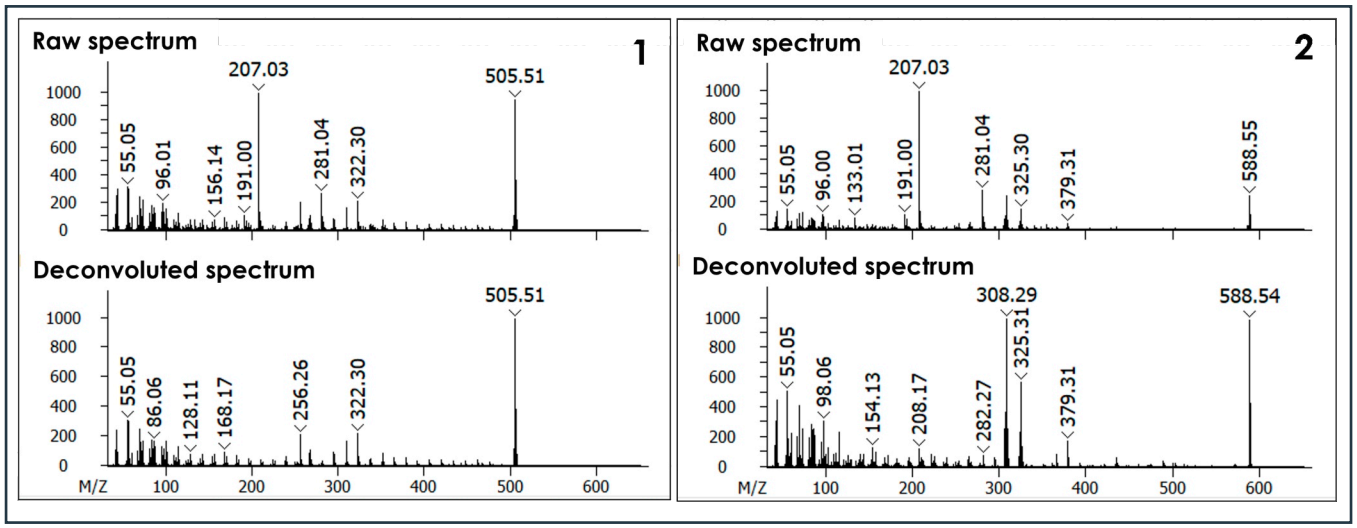


Figure 7. Raw (top) and deconvoluted (bottom) spectra for the unknown analytes observed in Sample D. Deconvolution provided cleaner spectra, yet neither matched to library databases.

For these cases, it is helpful to acquire and leverage additional spectral information. The *Pegasus* HRT$^+$ is a high-resolution accurate mass TOFMS system that can provide this additional detail. Thus, Sample D was analyzed with the *Pegasus* HRT$^+$ to further explore these two unknown features. In addition to improved resolution, the *Pegasus* HRT$^+$ has a multi-mode ion source (MMS™) that can be used to acquire both EI and CI on the same hardware, streamlining the investigation of these unknowns. This EI and CI data provided additional mass spectral information for formulae determination and spectral interpretation.

The formulae determinations, supported with excellent mass accuracy of both EI and CI data, and spectral interpretations for these unknowns are shown in Figures 8 and 9. As shown, these data provided information to tentatively propose structures for both of the unknowns. Oleyl palmitamide was proposed for the first unknown, shown in Figure 8. This analyte is a lubricant additive that is used as a slip agent and anti-blocking agent. The second unknown was proposed as N,N'-ethylenedioleamide, as shown in Figure 9. This analyte is a bisamide wax and anti-fogging agent. Standards for both of these analytes were purchased and analyzed, confirming the identifications.

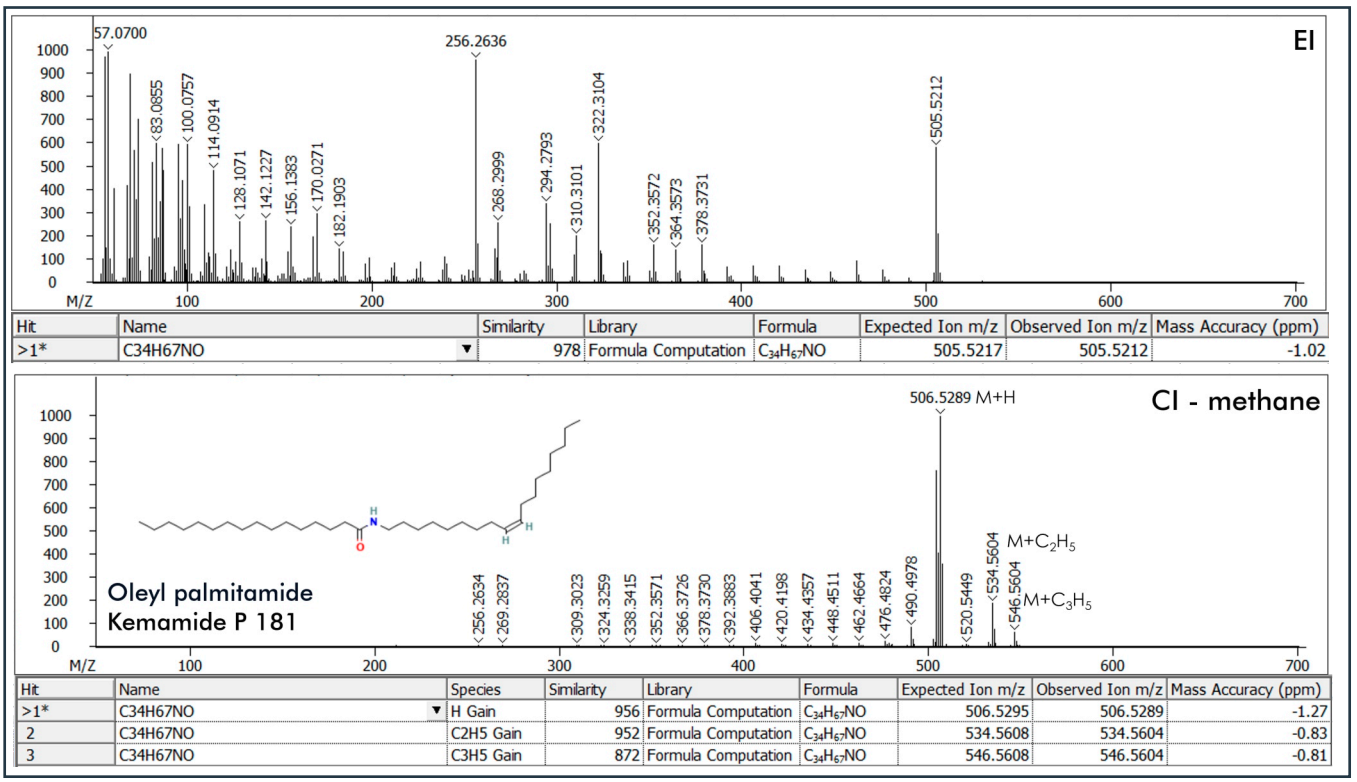
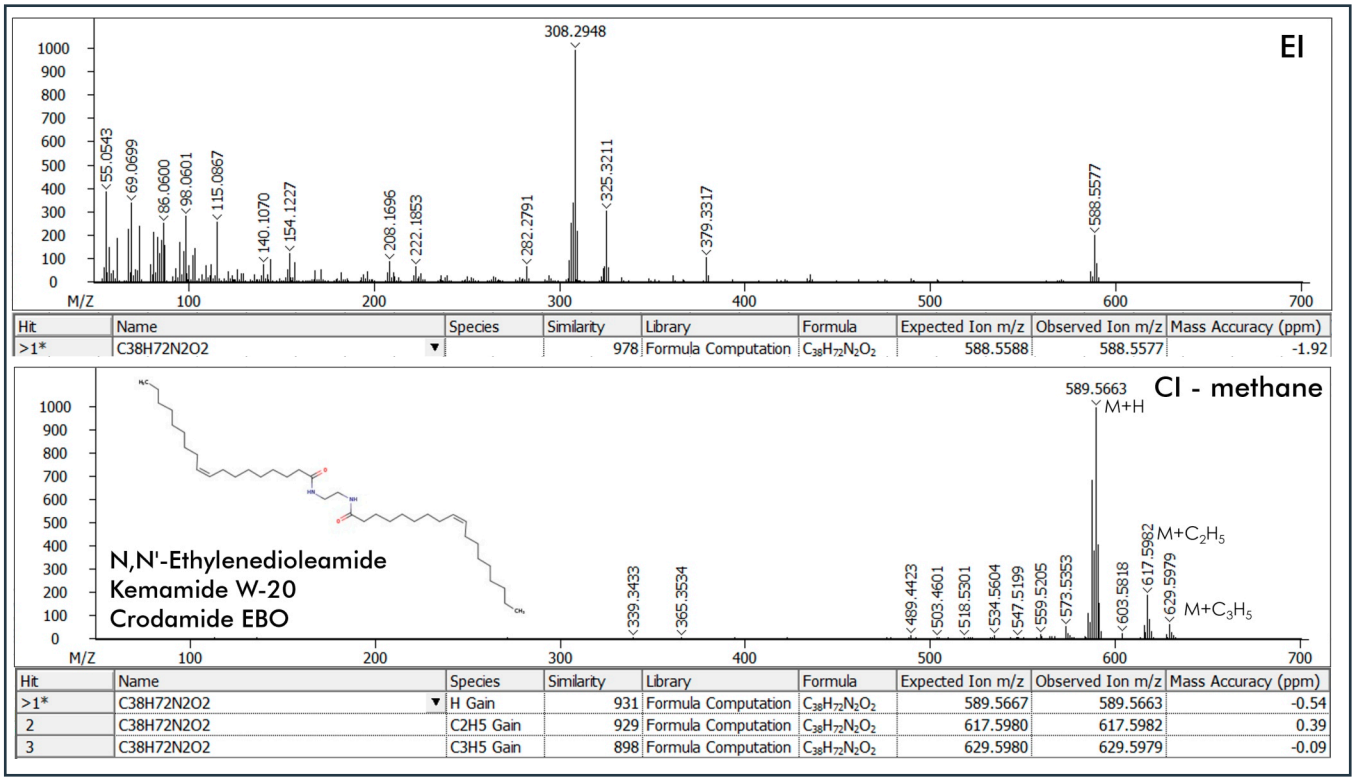


Figure 8. EI and CI data from the Pegasus HRT$^+$ for the first unknown peak in Sample D.



Hit	Name	Species	Similarity	Library	Formula	Expected Ion m/z	Observed Ion m/z	Mass Accuracy (ppm)
>1*	C38H72N2O2		978	Formula Computation	$C_{38}H_{72}N_2O_2$	588.5588	588.5577	-1.92

Hit	Name	Species	Similarity	Library	Formula	Expected Ion m/z	Observed Ion m/z	Mass Accuracy (ppm)
>1*	C38H72N2O2	H Gain	931	Formula Computation	$C_{38}H_{72}N_2O_2$	589.5667	589.5663	-0.54
2	C38H72N2O2	C2H5 Gain	929	Formula Computation	$C_{38}H_{72}N_2O_2$	617.5980	617.5982	0.39
3	C38H72N2O2	C3H5 Gain	898	Formula Computation	$C_{38}H_{72}N_2O_2$	629.5980	629.5979	-0.09

Figure 9. EI and CI data from the Pegasus HRT⁺ for the second unknown peak in Sample D, also observed in Sample B.

This collection of analytical tools provided detailed information on the extractables above the AET in the dental bite guard extracts, showing value for these aspects of the ISO 10993 workflow.

Conclusion

In this application note, extracts for over-the-counter dental bite guards were analyzed with GC-TOFMS according to ISO 10993. The AET was determined with known extractables standards and analytes exceeding the AET in each dental bite guard extract were evaluated. The *Pegasus* BTX and *Pegasus* HRT⁺ were both utilized for this workflow. All samples were screened with the *Pegasus* BTX and those with unknown analytes above the AET were further evaluated with the *Pegasus* HRT⁺. Identifications were determined with spectral matching to library databases and RI verification when possible. High-resolution TOFMS provided additional spectral data for interpretation, molecular formulae determinations, and tentative structure proposals. Standards for the proposed unknowns were also analyzed to support the identifications. These tools can be a useful part of these risk management strategies.

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