APPLICATION NOTE

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Characterizing unknowns in food packaging using GC Orbitrap Mass Spectrometry

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Key Words

Food packaging, Q Exactive GC, Orbitrap mass spectrometry, unknown identification, structural elucidation, food safety

Introduction

Packaging is an essential element of a safe food supply chain, with its main purpose to preserve the food it covers and to maintain its quality over the course of the products shelf life. Without an adequate barrier, food producers and manufacturers risk potentially serious microbial and chemical food safety incidents that may result in serious health risks over the short or long term. However, it is also well known that the chemical components used in the packaging can migrate into the food and present an even greater threat.[1] Food and beverages can interact strongly with any surface that they come into contact with and can potentially impact the quality of the product.[2] They can be corrosive or cause other physical breakdown of the packaging that will, in turn, leach chemicals into the product. Unfortunately, no packaging material is entirely inert; glass, paper, plastics and ceramics can all leach chemicals into the food at significant concentrations. For these reasons, it is important that regulators and manufacturers monitor and understand the health risk associated with packaging and take steps to minimize the risk to the consumer.

Gas Chromatography-Mass Spectrometry (GC-MS) is a popular analytical technique and has been widely used in food packaging studies as it provides analytical advantages of chromatographic resolution, reproducibility, peak capacity and, importantly, extensive spectral libraries to aid in identification. The analytes of interest are either volatile or semi-volatile (<1000 Da) in nature, and are therefore well-suited to analysis by GC-MS. The primary materials, such as monomers, additives and solvents used in the food packaging are usually well understood. However, these materials can also contain non-intentionally added substances (NIAS) such as impurities, reaction intermediates, breakdown products of polymer/additives, and contaminants from recycling.

When investigating NIAS in food packaging, the analysis is challenging because there is very little information of the potential chemicals involved. Therefore, the approach taken needs to be as non-selective as possible so that the maximum chemical information is captured. To achieve this, the sample extraction technique is generic and often involves simple liquid extraction and concentration. This is followed by analysis in full-scan to obtain wide coverage of a sample. When using nominal mass GC-MS instruments for unknown analysis the procedure can be complex, time consuming, and expensive as it takes longer to interpret the mass spectrum and the confidence in any proposed assignment is low. Furthermore, there is a need for improved sensitivity because currently there can be extensive sample preparation and pre-treatment to isolate and concentrate samples which adversely impacts on the time to result.

This study focused on the utilization of a new GC-MS system with high mass resolution performance and high mass accuracy for fast and confident identification of unknown compounds in food packaging. Prior to this work, some of the unknown compounds were initially detected using nominal mass instrumentation (single quadrupole GC-MS), but this proved limited in the ability to assign an elemental formula, structure, and confident compound identification. Full-scan and MS/MS high mass resolution experiments are important to achieve the selectivity and mass accuracy needed for confident elemental composition proposals, structural elucidation and discrimination of co-eluting compounds. These features, in combination with novel software algorithms for automated spectral deconvolution and compound ID, create a powerful solution for fast, confident and comprehensive chemical characterization of food packaging samples.

Experimental conditions

Sample preparation

The sample investigated in this study was a tin can with an internal coating. The internal coating was extracted using a 300 mL solution of hexane: acetone (1:1) held at room temperature for 16 hours. The 300 mL was then evaporated to approximately 1 mL before being transferred to a crimp cap amber GC vial for analysis.

Instrument and method setup

In all experiments a Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system and a Thermo Scientific™ TraceGOLD TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 10 m guard (P/N 26096-1421). Additional details of instrument parameters are displayed in Table 1 and Table 2.

Table 1. GC and injector conditions.

TRACE 1310 GC System Parameters	
Injection Volume (µL)	1
Liner	Single gooseneck P/N 453A0344-UI
Inlet (°C)	SSL 280
Carrier Gas, (mL/min)	He, 1.3
Oven Temperature Program	
Temperature 1 (°C)	40
Hold Time (min)	0.5
Temperature 2 (°C)	325
Rate (°C/min)	5.5
Hold Time (min)	12

Table 2. Mass spectrometer conditions.

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C)	280
Ionization type	EI/PCI
Ion source (°C)	230 EI / 190 CI
Electron energy (eV)	70
Acquisition mode	Full-scan
Mass range (Da)	50–700
Resolving power (FWHM at *m/z* 200)	120,000
Lockmass, column bleed *(m/z)*	207.03235

The Q Exactive GC system was operated in EI full-scan mode using 120,000 (FWHM at *m/z* 200) resolving power. Additional experiments were run using positive chemical ionization (PCI) with methane as reagent gas at a flow of 1.5 mL/min to obtain information on the molecular ions and to support the identification of unknown component peaks.

Data processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder also contains accurate mass spectral deconvolution and spectral matching functionality. Thermo Scientific™ MassFrontier™ spectral interpretation software was used for structural elucidation.

Results and discussion

The objective of this study was to analyze the packaging sample using a non-target full-scan data acquisition using electron ionization (EI) and positive chemical ionization (PCI), and to identify the most intense peaks. In addition, the aim was to provide structural information for the peaks detected using nominal mass GC-MS, where confirmation of the identity was not possible.

Extracting key features

Full-scan chromatograms were obtained for the sample and the total ion chromatograms (TICs) are shown in Figure 1. The Q Exactive GC system acquires accurate mass data with a wide dynamic range. This is very powerful when the objective is to identify unknown peaks in a complex sample, such as a food packaging extract with a high degree of confidence. The first step in this analysis was to isolate the peaks of interest and although peaks can be seen visually in the TICs, it is essential that all features are extracted from the data.

This was achieved with TraceFinder which first performs a high resolution accurate mass deconvolution of the data with the aim of detecting all of the peaks above a signal to noise threshold of 100:1. The deconvolution ensures that only ions that maximize at the same retention time remain for library matching. Using these thresholds, 961 features (peak clusters) were detected in the packaging sample. An example peak for 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde is shown in Figure 2, along with the number of scans across the peak, the accurate mass and ppm difference.

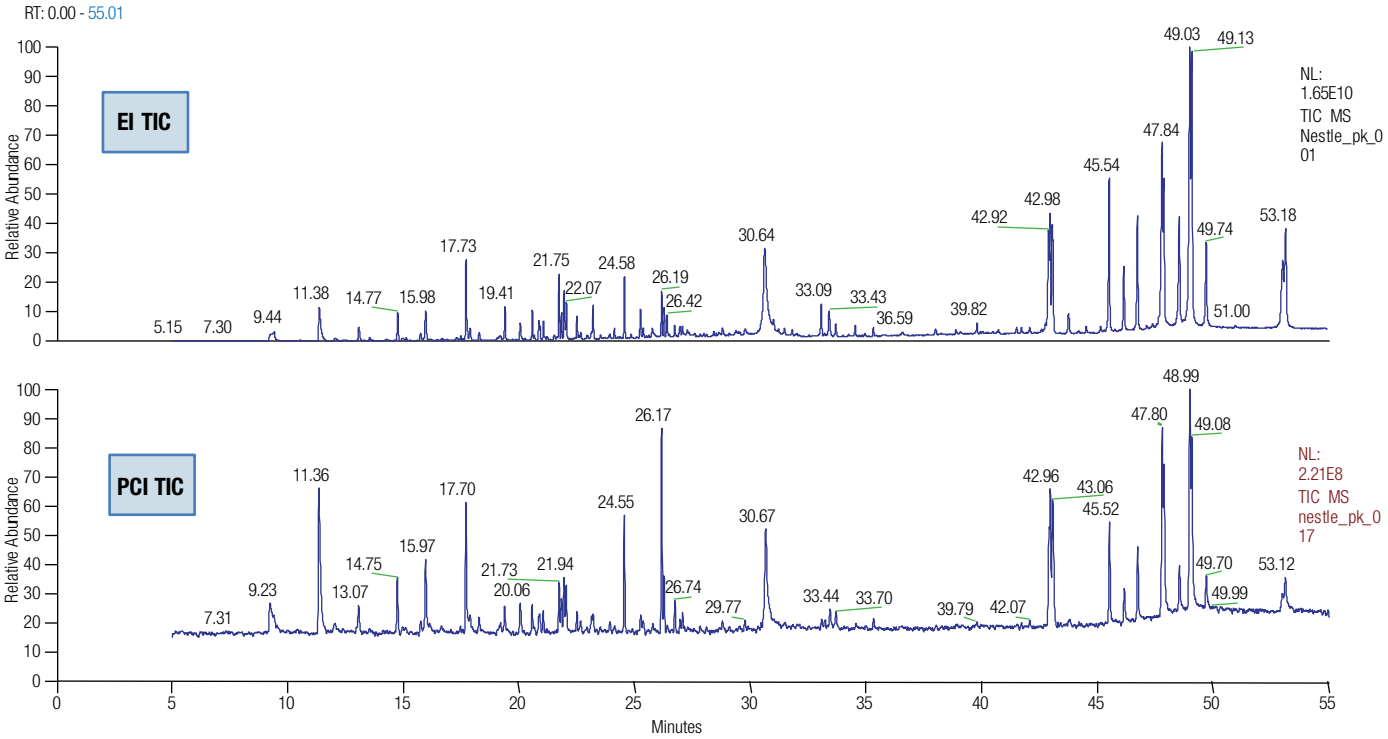

Figure 1. GC-MS electron ionization (EI) and positive chemical ionization (PCI) total ion chromatograms (TIC) of the packaging sample.

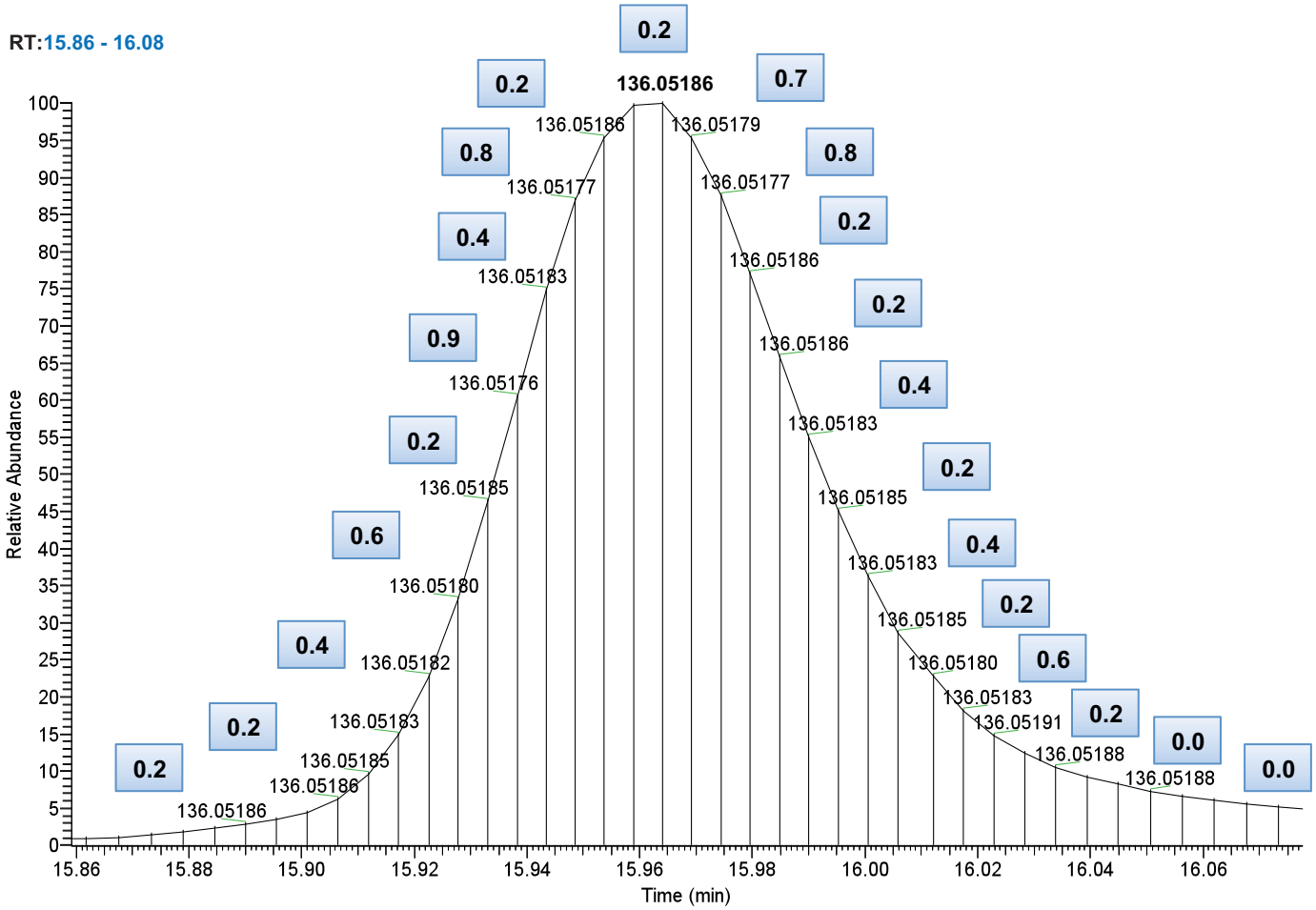

Figure 2. Extracted ion chromatogram for compound 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde fragment (m/z 136.05188 ±5 ppm mass window) in packaging sample 34 scans/peak. Data acquired in full-scan at 120,000 FWHM resolving power. Excellent accurate mass stability is shown for each individual scan as well as mass difference labelled (in ppm).

Accelerate known compound identification

Having performed a peak extraction, the deconvoluted spectrum was first searched against a commercially available nominal mass spectral library (NIST 2014). If available, the data could also be searched against an in-house nominal or accurate mass spectral library. The lists of hits were scored based on a combination of the search index (SI) score and high resolution filtering (HRF) value. The HRF value is the percentage of the mass spectrum that can be explained by the chemical formula in the library search.[3]

The combination of accurate mass and percentage of explained ions observed in the spectrum provides a fast and confident route to the identification of compounds. The utilization of accurate mass information speeds up the identification process as the user is no longer faced with long lists of spectral library matched compounds that are difficult to confirm or eliminate. For example, the top hit for the peak at 15.98 minutes was for the compound 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde, where 99.2% of the spectrum can be explained based on accurate mass (Figure 3). The fragments observed are matched to the elements in the proposed compound with sub 1 ppm mass accuracy which adds confidence in the identification. If only spectral matching was used, it would be difficult to confirm the identification.

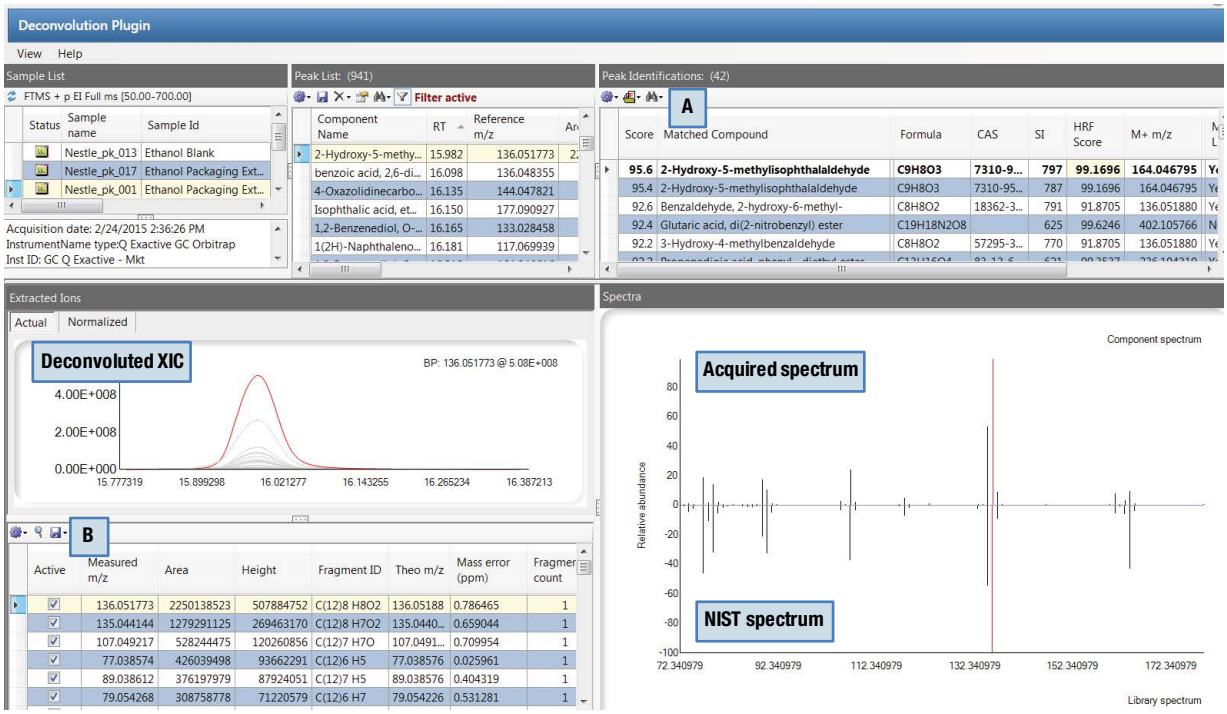

Figure 3. Identification of peak at 15.98 minutes as 2-hydroxy-5-methyl-1,3-benzenedicarboxaldehyde. Screenshot of the deconvoluted data and library match in TraceFinder. (A) List of library hits sorted by score (combination of SI and HRF). (B) List of fragment ions from EI spectrum and elemental composition based on elements in top hit.

Encountering unknowns

In a previous study, the same food packaging sample was analyzed using nominal mass GC-MS and a group of peaks were identified as being of interest, and they are also intense peaks in the high resolution MS TIC. These peaks eluted at RT: 30.6, 42.9, 45.5, 47.8, 49.1, and 53.2 minutes and are highlighted in Figure 4. As they are among the most intense peaks in the TIC, it is essential from a food safety view point to determine what they are as a first step to deciding whether they present any health risk.

Importantly, none of these peaks had a match in NIST 2014. With no spectral match it becomes extremely difficult using nominal mass to derive an acceptable degree of confident chemical compositional information about these compounds.

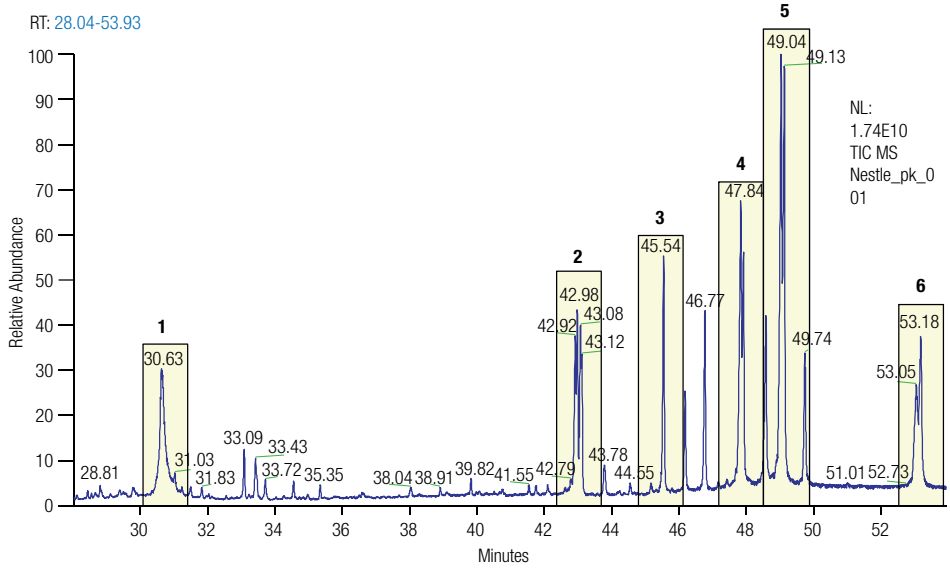

Figure 4. Zoomed region showing the six peaks of interest in the electron impact (EI) total ion chromatogram of the packaging sample.

When the spectral library match from the EI spectrum is inconclusive, then the PCI data can be used to establish the molecular ion, and to propose an elemental composition. When CI data is acquired using methane as the reagent gas, three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$. Figure 5 shows the EI and PCI spectra for the peak at 45.5 minutes. The PCI spectrum shows the adducts $[M+H]^+$ (-0.8 ppm) for ion *m/z* 469.18532, $[M+C_2H_5]^+$ (-0.5 ppm) for ion *m/z* 497.21677. The presence of these adducts indicated that the *m/z* 468.17783 was the molecular ion. Without the PCI adducts it would not be possible to determine if the *m/z* 468.17783 was a fragment or the molecular ion. From this ion, an elemental composition of the parent molecule can be proposed.

Elemental composition assignment is a critical stage in the compound identification process and it is where excellent mass accuracy and isotopic pattern can be used to limit the number of possible chemical formulae. An elemental composition calculator was used to propose a formula for the $[M+H]^+$ ion (Figure 6). The software assigns formulae by using an isotopic pattern matching algorithm that accounts for isotope accurate mass and intensity ratios. The algorithm uses a single mass to calculate all possible elemental compositions that lie within a tolerance window and then calculates the theoretical isotopic pattern for each suggestion. It then gives a score between 0 and 100 percent, where 0 is completely different and 100 an exact isotopic match. For example, when a 5 ppm mass accuracy window is used 12 possible formulae are proposed for the $[M+H]^+$ ion using the elements Carbon (1–30), Hydrogen (1–60), Nitrogen (1–5), Oxygen (1–10), Phosphorus (1) and Sulphur (1). This is compared to 1 ppm mass accuracy window that suggests three possible formulae. Only one of these suggestions has a 100 percent match with the theoretical isotopic pattern: $C_{26}H_{29}O_8$. This level of mass accuracy significantly reduces the number of formulae that need to be investigated, which speeds up the analysis, and also increases the confidence in any proposed assignment.

One final stage to support the proposed formula and to derive structural information is to use the accurate mass fragments. To achieve this, either the fragments in the EI spectrum can be used or an additional MS/MS experiment can be performed to be confident that the fragments are indeed from the molecular ion. The $[M+H]^+$ (PCI) *m/z* 469.18 was isolated in the quadrupole and fragmentation induced in the HCD cell using 15V energy.

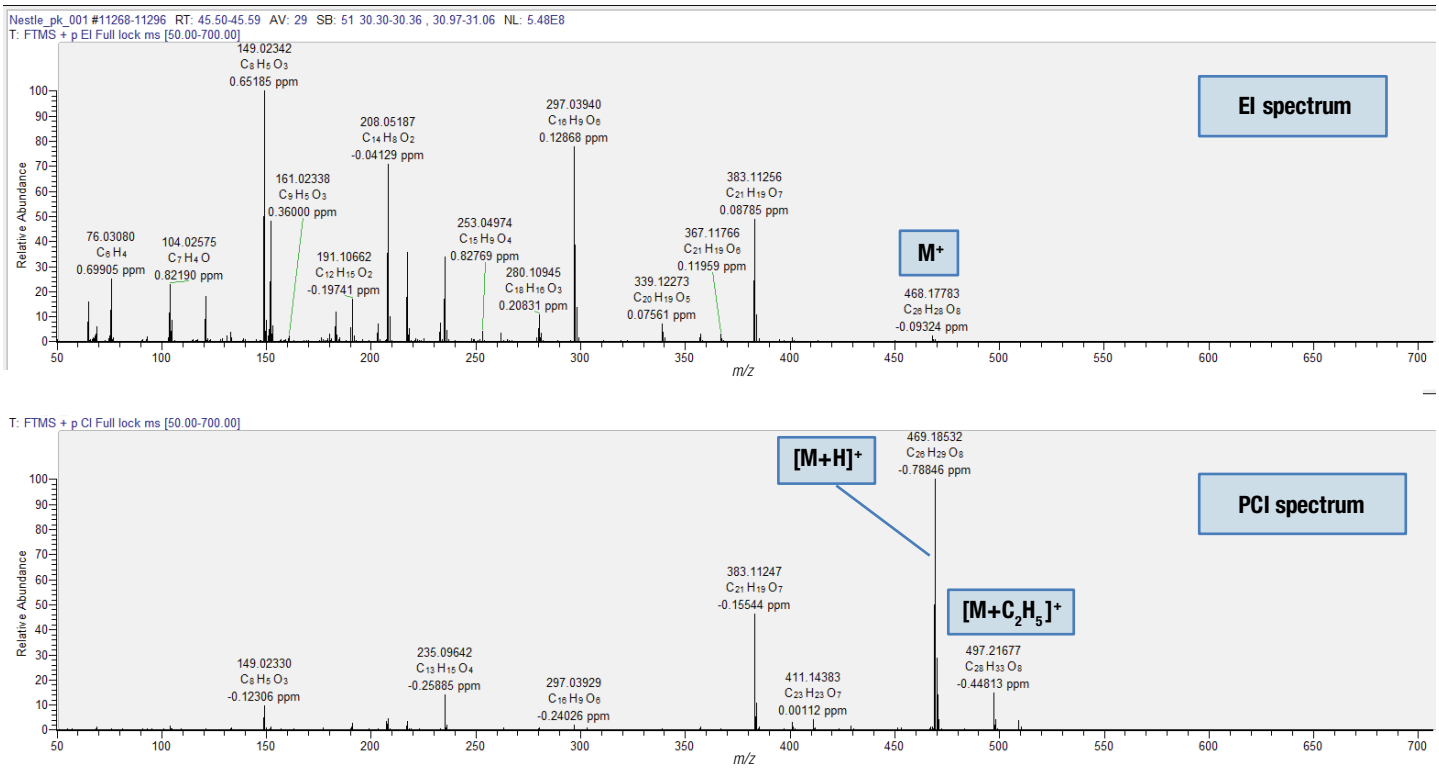

Figure 5. EI and PCI spectra at 45.5 minutes in packaging sample proposing a chemical formula of $C_{26}H_{28}O_8$. Peaks are annotated with chemical formula and mass difference in ppm. PCI data supports identification of parent ion with formula with sub 1 ppm mass accuracy.

Figure 7 shows the resulting MS/MS spectrum for *m/z* 469.18. The fragments measured contain the elements in the proposed parent and all with good mass accuracy. Based on this information, a proposed structure of the compound was made and is shown inset in Figure 7. MassFrontier was used to theoretically fragment the proposed chemical structure and match these to the measured fragments in the MS/MS spectrum. Therefore, even if at this stage a compound name cannot be confidently assigned, enough information can be obtained with respect to the chemical formula of the unknown compound.

Each of the six peaks were evaluated using the same workflow, and the results are summarized in Table 3. The mass accuracy obtained (<1 ppm) enabled confident elemental compositions to be assigned and these are supported by accurate mass fragments in the EI spectra. It was noted that all of the peaks contained a *m/z* 149.02332 ion and shared a common structure.

Figure 6. Elemental composition calculator screen in FreeStyle for the peak at 45.5 minutes in packaging sample proposing a chemical formula of $C_{26}H_{29}O_8$ for the $[M+H]^+$ ion based on accurate mass and isotope pattern. The three candidates are all within 1 ppm, but the top hit has a 100% isotopic match with the theoretical pattern.

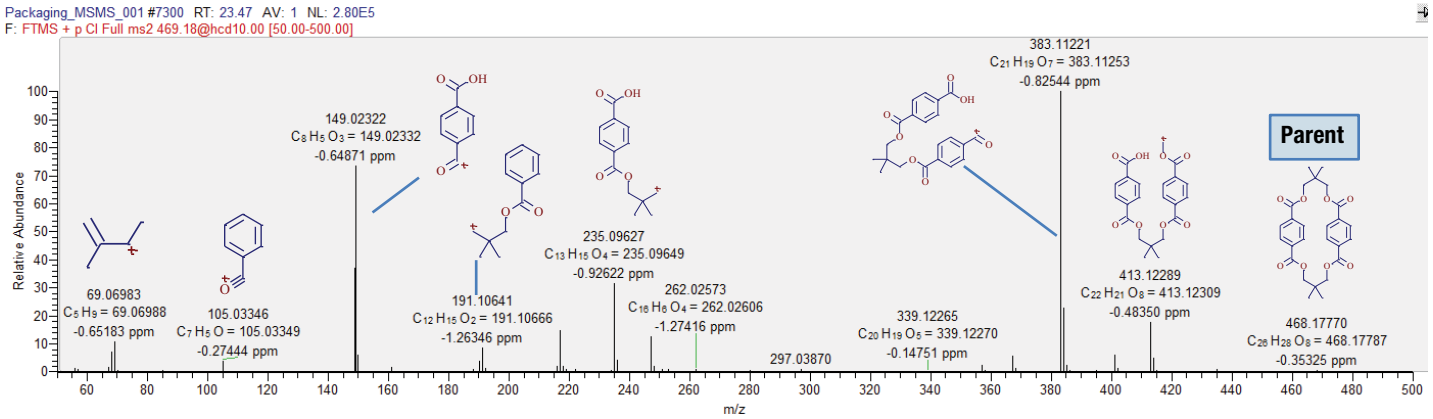

Figure 7. MS/MS spectrum of PCI ion *m/z* 469.18 selected in the quadrupole and fragmented in the HCD cell. MassFrontier used to explain the fragments observed within 3 ppm mass accuracy window.

Table 3. Summary of the peaks and the tentative identification of the elemental composition of the compounds. Excellent mass accuracy (<1 ppm) for all quasi-molecular ions adds confidence to the proposed identities.

Peak No.	Retention Time (min)	Formula	$[M+H]^+$ *m/z*	Mass Error of $[M+H]^+$ (ppm)	Mass Error of $[M+C_2H_5]^+$ (ppm)	Mass Error of $[M+C_3H_5]^+$ (ppm)
1	30.6	$C_{14}H_{18}O_6$	283.11762	0.0	0.5	0.1
2	42.98	$C_{22}H_{20}O_8$	413.12303	-0.2	-0.3	0.0
3	45.5	$C_{26}H_{28}O_8$	469.18532	0.7	-0.4	0.0
4	47.5	$C_{24}H_{24}O_8$	441.15424	-0.4	-0.4	-0.3
5	49.1	$C_{27}H_{30}O_8$	483.20112	-0.5	-0.1	0.3
6	52.0	$C_{28}H_{32}O_8$	497.21684	-0.3	0.1	0.3

Unlocking structural information

Further investigation of the full-scan EI and PCI data showed that when the parent mass for $C_{26}H_{28}O_8$ was extracted there were three peaks in the chromatogram (Figure 8). The capability to perform accurate mass MS/MS experiments provides valuable structural information that may be vital in determining what the compound is and if it is a safety concern. The MS/MS spectra for the three isomers (Figure 9) shows both similarities and differences between the isomers. Isomers 2 and 3 have a base peak at *m/z* 401.12309 ($C_{21}H_{21}O_8$) and an additional ion *m/z* 132.02058 ($C_8H_4O_2$). The base peak in isomer 1 is *m/z* 383.11253 ($C_{21}H_{19}O_7$) and the *m/z* 132.02058 is absent. The capacity to confidently assign elemental compositions to these ions is highly beneficial and provides the analyst with a complete picture. The *m/z* 401.12309 corresponds to a loss of C_5H_7 from the parent and *m/z* 383.11253 a loss of $C_5H_{10}O$. MassFrontier was used to explain how these ions can be derived from the proposed chemical structure. From this information, the flexibility to perform MS/MS experiments with accurate mass information allows for detailed structural information to be determined.

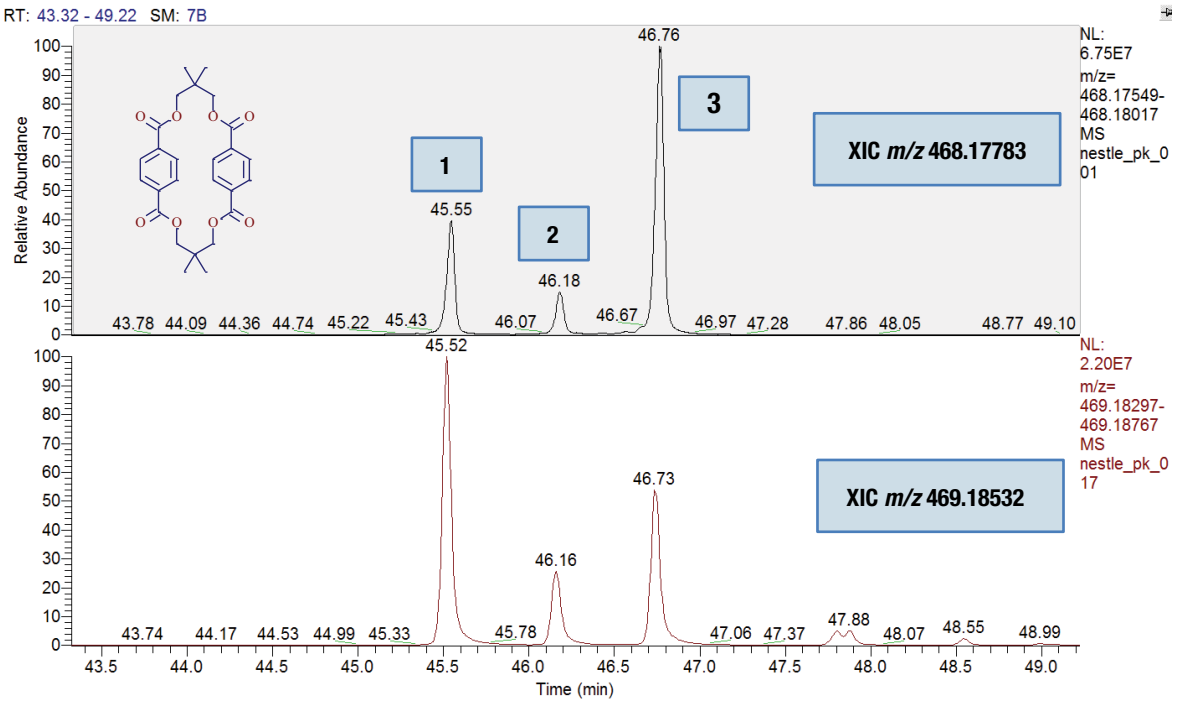

Figure 8. XIC *m/z* 468.17783 from the full-scan EI data and *m/z* 469.18532 from the full-scan PCI data in packaging sample shows 3 isomers of the same parent mass. Inset proposed chemical structure of compound.

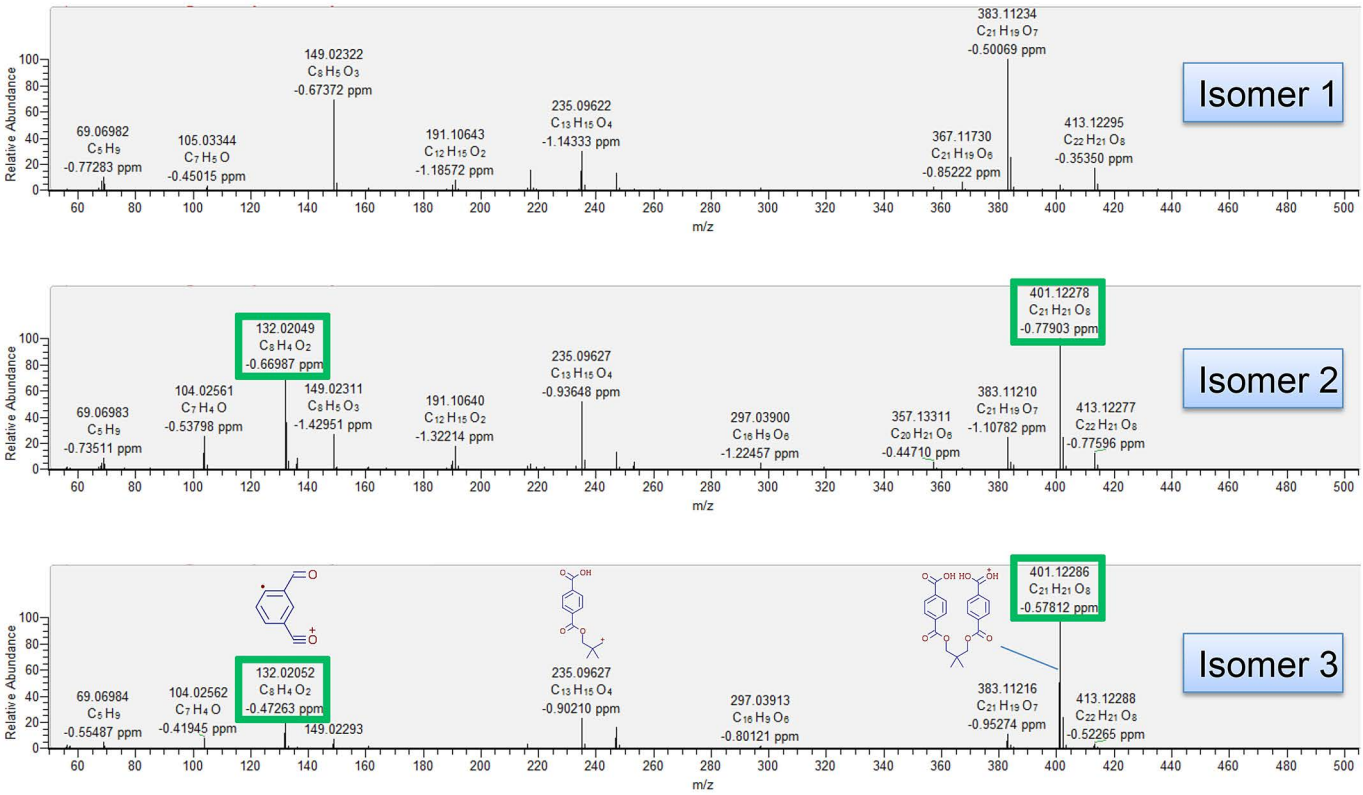

Figure 9. MS/MS spectra of m/z 469.18 of the three isomers reveals different fragmentation patterns for isomers 2 and 3. Of particular note, the base peak is 401.12286 and the presence of m/z 132.02049 ion.

Conclusions

The results of this study demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with easy-to-use software tools, is a powerful tool for the profiling of complex samples and for the identification of unknown chemicals. The Orbitrap mass spectrometer delivers excellent resolution and mass accuracy which leads to fast and confident characterization of samples regardless of the concentration. A food packaging sample was quickly screened for known compounds using spectral matching and rationalisation using accurate mass. EI and PCI information leads to confident chemical formulas to be proposed for molecular ions and fragments for compounds with no library match. Furthermore, the ability to perform high resolution, accurate mass MS/MS experiments completes the unknown identification workflow and allows for an even higher level of confidence and provides important structural information.

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