Application note | 001607

Food safety

# Untargeted screening and identification of substances in plastic food contact materials using an Orbitrap Exploris GC 240 mass spectrometer


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## Keywords

Orbitrap Exploris GC 240 mass spectrometer, mass resolving power, sensitivity, mass accuracy, Orbitrap technology, gas chromatography, NIAS, food contact materials, Compound Discoverer software


## Goal

The objective of this application note is to demonstrate the utility of gas chromatography-Orbitrap™ mass spectrometry and Thermo Scientific™ Compound Discoverer™ software for the detection and identification of non-intentionally added substances (NIAS) in a plastic film sample.

## Introduction

In the European Union (EU), plastic materials and articles intended to come into contact with food should comply with the Commission Regulation (EU) No 10/2022 and amendments.[1] This regulation contains a list of authorized monomers, other starting substances, macromolecules obtained from microbial fermentation, additives, and polymer production aids (intentionally added substances, IAS) that can be used for the manufacture of plastic food contact materials (FCM). However, during the manufacturing processes and uses of plastic FCM, the reaction and degradation of products can occur, leading to the formation of other compounds (non-intentionally added substances, NIAS) in the plastic material. For this reason, the risk associated with the presence and potential release of NIAS should be assessed before the authorization of FCM.[2]

Due to the wide range of volatility and polarity of such NIAS chemicals, different chromatographic techniques are used to undertake a comprehensive study. Most reported methods for untargeted analysis of plastic FCM are based on liquid

chromatography (LC) coupled to high resolution accurate mass (HRAM) mass spectrometry. For the volatile and semivolatile components, gas chromatography, often equipped with a headspace sampler and coupled to mass spectrometry (headspace GC-MS), is used to analyze the volatile fraction of migrated substances/chemicals. In this characterization phase, HRAM MS offers numerous advantages over nominal mass MS for the identification of unexpected substances, as the molecular formula of the acquired ions can be reliably obtained from their exact mass. In addition, HRAM brings higher selectivity than nominal mass GC-MS so that more features are detected and subsequently identified for further risk assessment. Where an unknown compound is encountered, HRAM, such as Orbitrap MS, provides high quality accurate mass data using either electron ionization (EI) or chemical ionization (CI) to identify molecular ions and perform fragmentation studies using MS/MS and quantitation to determine analyte concentration. With such high quality and comprehensive data, it is essential that intuitive informatics exist to extract all features, visualize results, and derive meaningful conclusions. For this aspect, Compound Discoverer software can be used to perform chemical migration studies from feature detection to statistical comparison and compound identification all within a single software environment.

The objective of this study was to develop an analytical method for the tentative identification of unknown migrant substances (IAS/NIAS) in plastic FCM by GC Orbitrap MS and to demonstrate the benefits of Compound Discoverer software for this application.

## Experimental

### Sample preparation

The sample analyzed in this study was a post-consumer recycled low-density polyethylene (LDPE) film. For the solvent extraction, a square piece (5 cm × 5 cm) was prepared. It was placed in a 50 mL glass beaker with 20 mL of acetone and incubated at 40 °C for 1 hour. The beaker was sealed with aluminum foil to prevent solvent evaporation. After the extraction, the whole volume of acetone was transferred to a second glass beaker and evaporated to a final volume of approximately 0.5 mL using a gentile nitrogen stream. The concentrated extract was transferred to a 1 mL volumetric flask and filled up to the mark with acetone. This solution was transferred into an injection vial. The same steps were applied to prepare a procedural blank containing no sample. Both samples were injected three times in the full scan electron ionization (EI) mode. Additionally, the polymer extract was injected using positive chemical ionization (PCI) mode to derive confirmation of molecular ion. To obtain the retention index (RI) values, a C8-C40 *n*-alkane mix was injected.

### Acquisition method

The analysis was performed with a Thermo Scientific™ TRACE™ 1610 GC coupled to a Thermo Scientific™ Orbitrap™ Exploris™ GC 240 mass spectrometer. All instrumental parameters are shown in Tables 1–3.

**Table 1. Parameters of the TRACE 1610 GC**

| TRACE 1610 GC | |
|---|---|
| **Injector** | |
| Injection volume (µL) | 1 |
| Inlet liner | Thermo Scientific™ LinerGOLD™ splitless liner, single taper with quartz wool (P/N 453A1925-UI) |
| Inlet temperature (°C) | 280 |
| Inlet module and mode | SSL, Splitless |
| Splitless time (min) | 1 |
| Septum purge flow (mL/min) | 5 |
| **Oven and column** | |
| Carrier gas, flow rate (mL/min) | He, 1.2 |
| Column | Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm i.d. × 0.25 µm (P/N 26096-1420) |
| **Oven temperature program** | |
| Temperature 1 (°C) | 40 |
| Hold time (min) | 5 |
| Temperature 2 (°C) | 315 |
| Rate (°C/min) | 5 |
| Hold time (min) | 10 |
| Total GC run time (min) | 70 |

**Table 2. Parameters of the Orbitrap Exploris 240 GC in electron ionization mode**

| Orbitrap Exploris GC 240 mass spectrometer in EI mode | |
|---|---|
| Transfer line (°C) | 300 |
| Ion source (ionization type) | Thermo Scientific™ ExtractaBrite™ (EI) source |
| Ion source (°C) | 280 |
| Electron energy (eV) | 70 |
| Emission current (µA) | 50 |
| Acquisition mode | Full scan (FS) |
| Mass range *(m/z)* | 40–500 |
| Orbitrap resolution | 120,000 |
| AGC target | Standard |
| Maximum injection time | Auto |
| Lock masses | 133.01356; 207.03235; 225.04292; 281.05114; 299.06171; 355.06993 |

**Table 3. Parameters of the Orbitrap Exploris 240 GC in positive chemical ionization mode**

| Orbitrap Exploris GC 240 mass spectrometer in PCI mode | |
|---|---|
| Transfer line (°C) | 300 |
| Ion source (ionization type) | ExtractaBrite (CI) |
| Ion source (°C) | 250 |
| CI gas | Methane |
| CI gas flow | 1 |
| Acquisition mode | Full scan/ddMS$^2$ |
| FS Mass range (*m/z*) | 100–700 |
| FS Orbitrap resolution | 120,000 |
| FS AGC target | Standard |
| FS Maximum injection time | Auto |
| ddMS$^2$ Scans | 5 |
| ddMS$^2$ Filters | Dynamic exclusion, Apex detection |
| ddMS$^2$ Isolation window (*m/z*) | 1.2 |
| ddMS$^2$ HCD collision energies (V) | 30 |
| ddMS$^2$ Orbitrap resolution | 15,000 |
| ddMS$^2$ Scan range | Auto |
| ddMS$^2$ AGC target | Standard |
| ddMS$^2$ Maximum injection time | Auto |

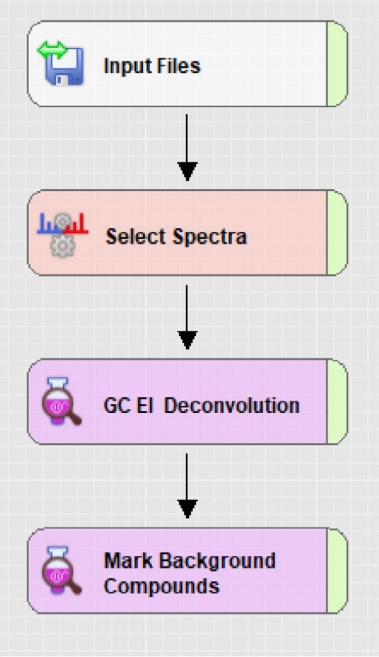



**Figure 1. The EI workflow in Compound Discoverer employed to identify the compounds present in the extract.**

## Data processing

In the first stage of data processing, the full scan EI results were evaluated using the workflow outlined in Figure 1. The workflow included spectral deconvolution followed by spectral matching with the NIST™ 2020 library. The "Mark Background Compounds" node was added to simplify further data analysis. This node compares the analytical sample with the blank. A compound that has

$$\frac{\textit{peak area in sample}}{\textit{peak area in blank}}$$

below a desired threshold (5 by default) is marked as a background compound and can be hidden in the results table.

In the investigated sample, a total of 260 features were detected (after removal of background compounds), 186 of them were identified with NIST library search, of which 150 obtained the Total Score above 90%. Total Score is a parameter that includes NIST search index (SI) and NIST reversed search index (RSI) as well as High Resolution Filtering (HRF) and Reverse High Resolution Filtering (RHRF). The latter two reflect the percentage of the spectrum that is explained by the chemical formula proposed from the best library hit. Additionally, for the compounds that had an RI value in the NIST library, a criterion of $\Delta RI < 50$ was applied. That reduced the number of identified compounds to 112.

Figure 2 shows the Compound Discoverer software workflow applied for the PCI data processing. The PCI data were used to confirm the tentative EI identification. Chemical ionization is much softer than EI, thus the molecular ion can be easily detected. The PCI workflows are based on the presence of molecular ion. In this study the following identification nodes were applied:

- Predict Composition: predicts the chemical formulas of the unknown compounds
- Search ChemSpider: performs a search in the ChemSpider™ databases
- Search mzCloud: performs a search in the mzCloud™ spectral library
- Search Mass List: serves to a databases search (this node is also available for EI workflows)

The task of the Assign Compound Annotations GC CI node is to assign and to prioritize the annotation coming from the nodes (Predict Composition, Search ChemSpider, Search mzCloud, and Search Mass List). In Compound Discoverer software, there is also a node that enables search in Thermo Scientific™ mzVault™ libraries, however in this study it was not employed.

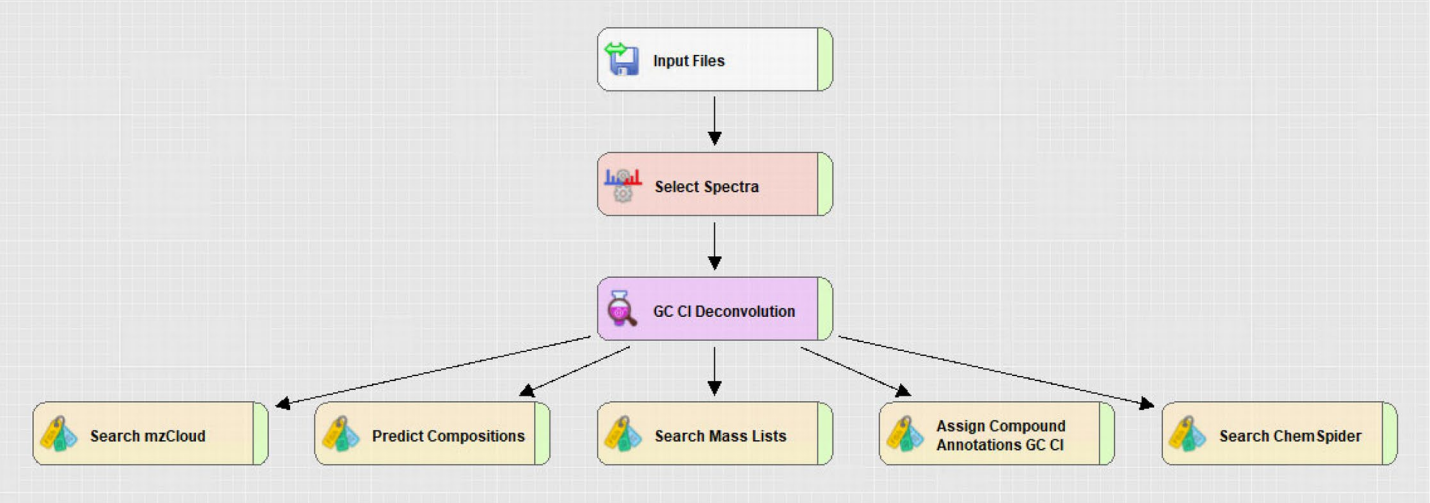



**Figure 2. The PCI Compound Discoverer software workflow used to confirm the compounds identified in the EI workflow.** All nodes can be customized depending on which library or identification route the user chooses to use.

## Examples of identification and confirmation

Methyl palmitate was identified with a Total Score of 95.4% and ΔRI 4, as demonstrated in Figure 3a (only data entries relevant to this compound are shown). The total number of the rows was 260 (detected features without the features present in the blank). Beside the Total Score, SI, RSI, HRF, and RHRF (all of them explained above) the results table contains:

- Name: this name comes from the NIST library, however it can be edited
- Calc. MW: neutral mass expressed in Da
- RT: retention time
- Reference *m/z*: *m/z* used to calculate peak area as well as to draw the extracted ion chromatogram (Figure 3b)
- Avg TIC: average TIC calculated for all the replicates
- NIST Lib Hit Formula: molecular formula associated to the NIST hit
- NIST Observed Mol. Mass: exact mass of the molecular ion (if present)
- Calculated RI: calculated retention index
- RI delta: delta between calculated RI and the RI present in the NIST library
- Group areas: peak area calculated as a median form all replicates
- Group CV: coefficient of variance calculated from all replicates

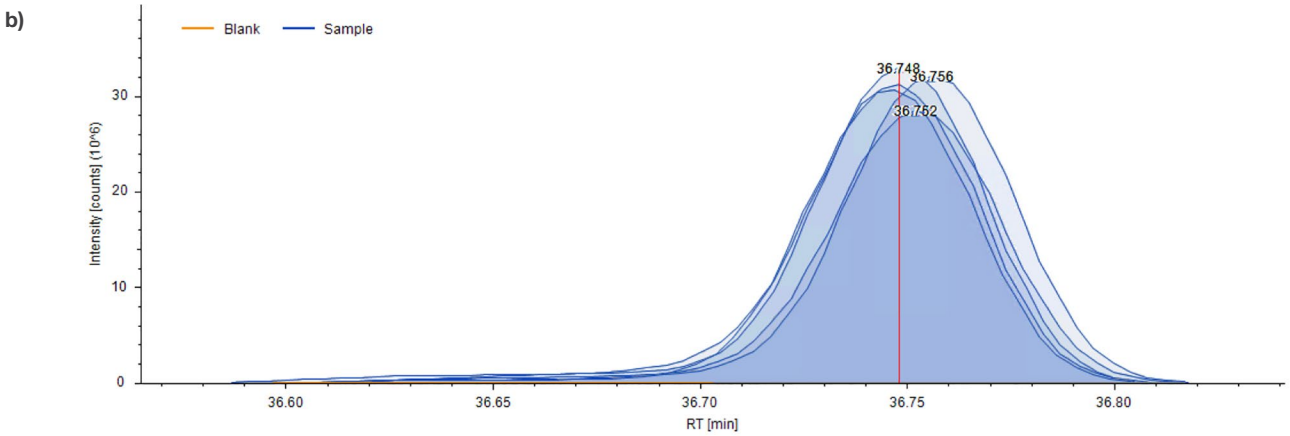
Any of these columns can be hidden if not necessary for the analyst. Figure 3b shows the extracted ion chromatograms of the reference ion, where all replicates are visible. Figure 3c demonstrates a comparison of the deconvoluted spectrum with the spectrum present in the NIST library. Although Compound Discoverer software is designed to process high-resolution data only, it is fully compatible with nominal mass NIST libraries. Methyl palmitate was identified against this library; therefore, the reference spectrum shows only zeros in the decimal places. In the discussed case, the molecular ion was detected, hence the corresponding molecular formula, exact mass, and structure are depicted.

To confirm the identification, the PCI data were revised. Figure 4 shows the PCI MS spectrum at the retention time corresponding to the peak identified in EI as methyl palmitate. Compound Discoverer software found various forms/adducts of the methyl palmitate molecular ion ($[M\text{-}H^-]^+$; $[M+H]^+$; $[M\text{-}e]^+$; $[M+C_2H_5]^+$; $[M+C_3H_5]^+$). Moreover, the isotopic pattern corresponding to the protonated molecule of methyl palmitate was observed.

In the PCI acquisition method, the full scan MS was combined with a data dependent $MS^2$. This means that every full scan spectrum was followed by the $MS^2$ analysis of the five most abundant ions in that spectrum. When working with the Orbitrap Exploris GC mass spectrometer, the user can choose the number of the precursor ions that will be fragmented in the $ddMS^2$ experiments; the minimum is 1, while the maximum is 100. The sample investigated here was relatively clean, thus five $MS^2$ experiments per one full scan spectrum were sufficient.

a)

| Name | Calc. MW | RT [min] | Reference m/z | Avg TIC | NIST Lib Hit Formula | NIST Theo. Mol. Mass | NIST Observed Mol. Mass | Total Score | HRF Score | SI | Calculated RI | RI Delta | Group Areas | Group CV [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl palmitate | 270.25589 | 36.748 | 55.05414 | 27334199 | C17 H34 O2 | 270.25533 | 270.25534 | 95.4 | 99.0 | 792 | 1930 | 4 | 1.05e8 | 6 |



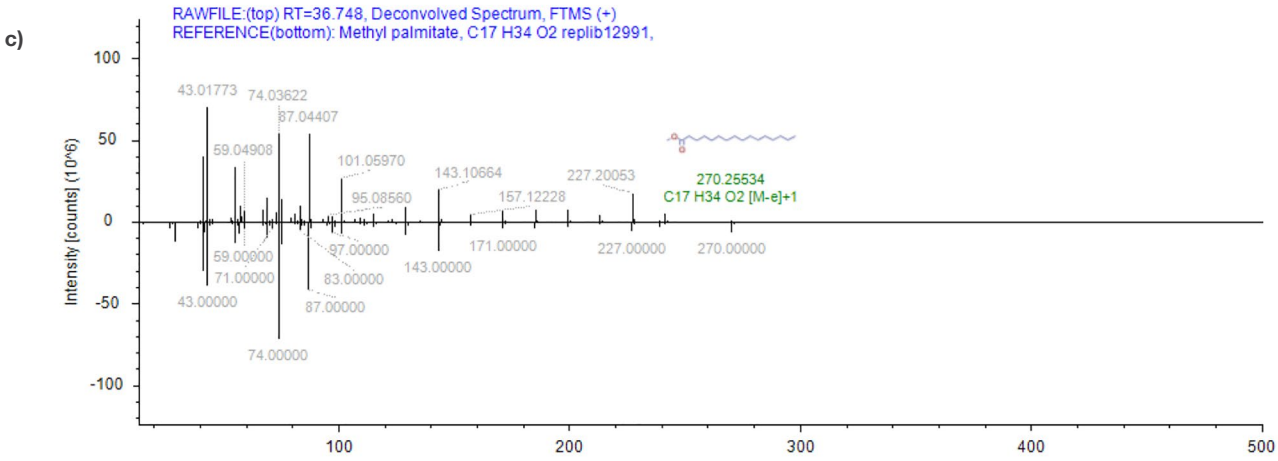




Figure 3. Identification of methyl palmitate in the EI workflow. a) the corresponding row from the results table, b) extracted ion chromatogram of the reference ion, c) comparison between deconvoluted spectrum (top) and NIST spectrum (bottom).

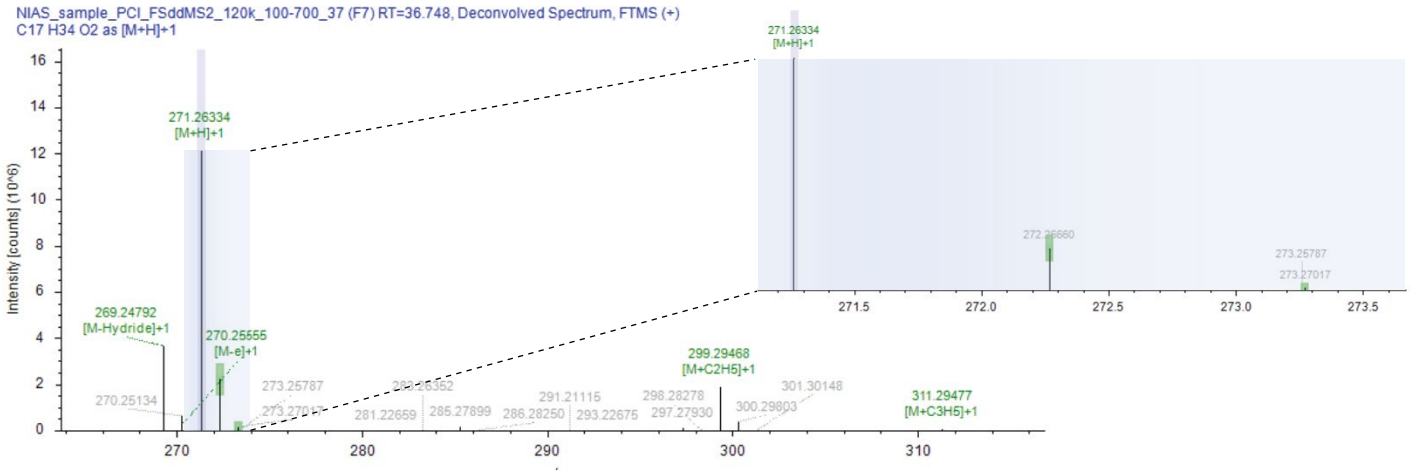


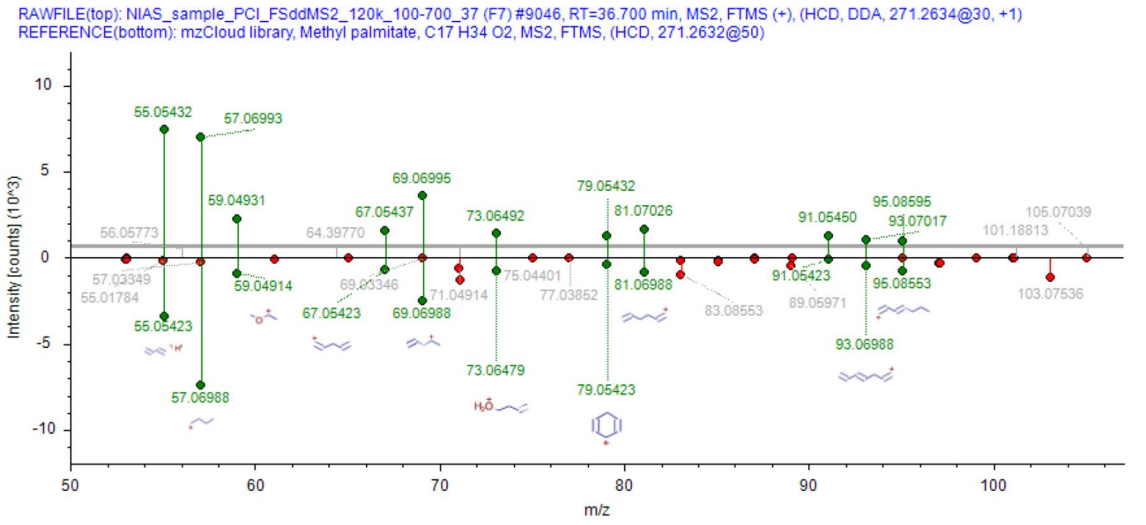

**Figure 4. PCI MS spectrum.** The molecular ions and isotopic pattern of methyl palmitate are marked automatically by the software in green.

The availability of the $MS^2$ data provided additional confirmation tools. A search in $MS^2$ libraries and databases is the most straightforward way to utilize $MS^2$ scans. In this study, an mzCloud library search was carried out. The mzCloud library is an exact mass library that contains both MS as well as $MS^2$ data. Fragmentation data present in the mzCloud library were acquired with multiple collision energies. Therefore, users can acquire data with any collision energy and do not have to modify existing methods to make them compatible with the library. Currently, the number of spectra present in the mzCloud library is approaching 10,000,000 across both LC- and GC-MS. Figure 5 shows a comparison between experimental $MS^2$ spectrum of the compound detected at *m/z* 271.2634 (presumably protonated methyl palmitate) and a methyl palmitate $MS^2$ spectrum present in the mzCloud library. The table directly below the spectra shows that the match was 85.9%.

Although the $MS^2$ spectrum was available for methyl palmitate, this may not be the case for all substances. In such cases the FISh scoring function can be very helpful.The FISh scoring is applied to explain the fragments in the fragmentation spectra based on *in silico* fragment prediction. The algorithm compares the experimental fragmentation spectra for a compound to the expected fragmentation spectra based on the structure of the compound. Moreover, it annotates the centroids in the fragmentation scans with the matching fragment structures.

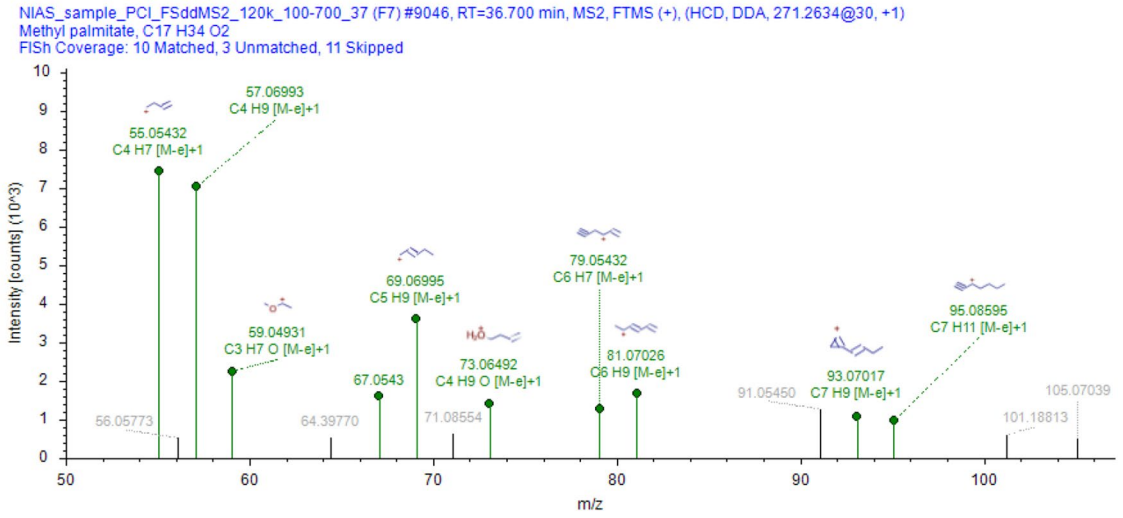
A structure for the *in silico* fragmentation can be drawn in Compound Discoverer software or it can be imported directly from ChemSpider or from a file (*.mol, *.mcs). What should be highlighted here is the fact that FISh is more than a simple tool that only “cuts” the molecules. The algorithm takes into account possible structural rearrangements that can take place during collision induced dissociation. Figure 6 shows a FISh scoring output. The algorithm was able to explain 76.92% of the ions present in the spectrum and highlighted in green color. Proposals of structures are depicted above the explained ions.

Methyl palmitate was present in the mzCloud library; thus application of FISh scoring was not so critical. In the case of methyl dehydroabietate (methyl abieta-8,11,13-trien-18-oate), identified in the EI and confirmed in the PCI mode by detection of various adducts and expected isotopic pattern, no match was found in the mzCloud spectral library. Thus, the only way to take advantage of the $MS^2$ data was to apply *in silico* fragmentation (Figure 7). Although, the FISh coverage is slightly lower than in the case of methyl palmitate, it worth noticing that non-explained ions have low intensity. Moreover, many of the explained ions have *m/z* > 100, which means that they are very selective, and they provide high confidence in the identification.



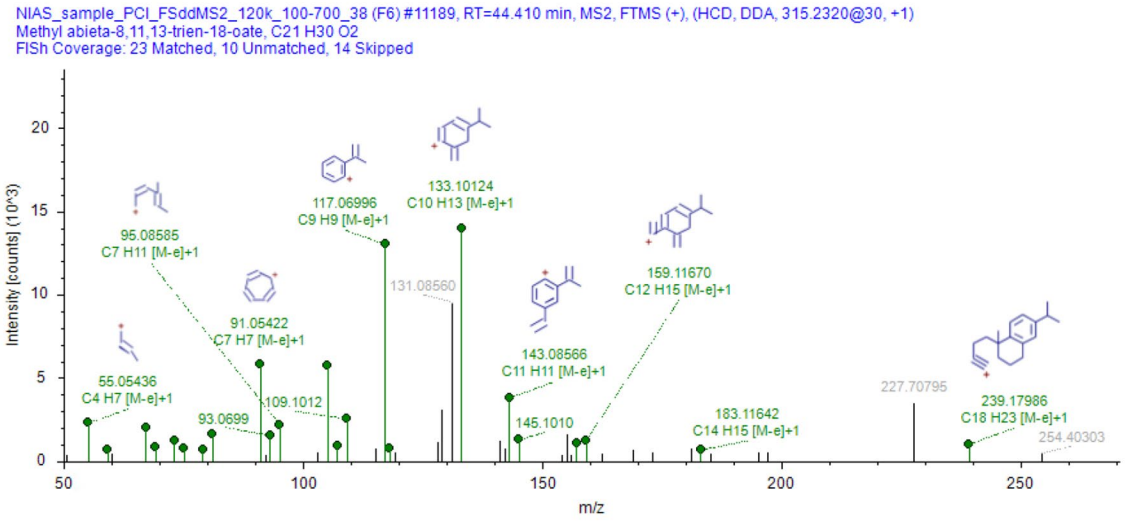

| Structure | Name | Formula | Molecular Weight | Best Match |
|---|---|---|---|---|
|  | Methyl palmitate | C17 H34 O2 | 270.25588 | 85.9 |

**Figure 5. Comparison between experimental $MS^2$ spectrum (top) and mzCloud MS spectrum (bottom) for methyl palmitate**




| Structure | Name | Formula | Molecular Weight | FISh Coverage |
|---|---|---|---|---|
|  | Methyl palmitate | C17 H34 O2 | 270.25588 | 76.92 |

Figure 6. MS$^2$ spectrum of methyl palmitate with ions explained by *in silico* fragmentation



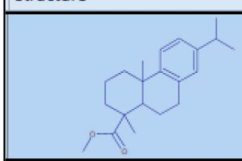

| Structure | Name | Formula | Molecular Weight | FISh Coverage |
|---|---|---|---|---|
|  | Methyl abieta-8,11,13-trien-18-oate | C21 H30 O2 | 314.22458 | 69.70 |

Figure 7. MS$^2$ spectrum of presumably methyl dehydroabietate with ions explained by *in silico* fragmentation

## Summary

Compound Discoverer software is an excellent option for the analysis of packaging migration substances. The "Mark Background Compounds" node can hide compounds present in the blank so that the analyst can clearly see the peaks of interest. Consequently, data revision is faster and less complicated. The combination of EI and PCI workflows provides numerous tools for compound identification and confirmation. The EI workflows are compatible with both nominal and exact mass NIST libraries, which facilitates identification of the broadest possible range of substances. The EI identification can be easily confirmed in the PCI mode, which involves MS as well as $MS^2$ data analysis. The MS nodes are focused on the molecular ion and information that is provided by this species (molecular formula prediction, isotopic pattern analysis, ChemSpider search, mass list search), whereas the $MS^2$ data can be used for the mzVault library search, mzCloud search, and FISh scoring. The latter is very helpful in cases where there are no library hits/matches. The FISh algorithm performs *in silico* fragmentation to predict the fragmentation products from the precursor ion structure.

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