Application Note 02126

Analysis of Vulcanized Rubber Formulations on the Varian 500-MS Ion Trap Mass Spectrometer and Confirmation of Expected Structures Using Varian TurboDDS™ with ACD/MS Manager Software

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Introduction

Vulcanization refers to a specific curing process of rubber involving high heat and the addition of sulfur or other equivalent curatives. It is a chemical process in which polymer molecules are linked to other polymer molecules by atomic bridges composed of sulfur atoms or carbon-to-carbon bonds. The end result is that the springy rubber molecules become cross-linked to a greater or lesser extent. This makes the bulk material harder, much more durable and also more resistant to chemical attack. It also makes the surface of the material smoother and prevents it from sticking to metal or plastic chemical catalysts.

The complexity and number of compounds generated makes the outcome of a vulcanization process unpredictable. Liquid chromatography/mass spectrometry (LC/MS) is an excellent tool to separate and characterize the molecular weight of all of the ionizable components in extracts of different rubber formulations. The addition of MS/MS allows for structural elucidation and the confirmation of expected compounds, polymeric end products and suspected contaminants, using specialized software.

In this method, an extract of vulcanized rubber is analyzed. The polymeric component is identified by evenly spaced clusters of mass spectral peaks. In addition, a chemical component is suspected to be found in the rubber extract. It is thought to be an antioxidant used in the vulcanization of the rubber, Antioxidant 2246, 2,2'-Methylenebis (4-methyl-6-tert-butylphenol). See structure in Figure 1. The ACD/MS Manager software can be used to confirm the presence of this compound based upon MS/MS fragmentation data obtained using Turbo DDS™ software on the 500-MS Ion Trap Mass Spectrometer.

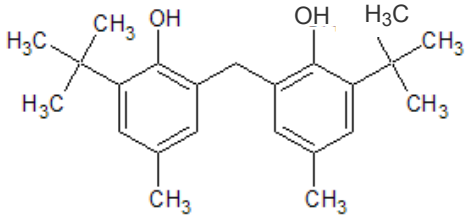

Figure 1. Chemical structure for Antioxidant 2246, suspected to be present in the vulcanized rubber extract, molecular weight 340.4 u.

Instrumentation

- Varian 212-LC Binary Gradient LC/MS pumps
- Varian 500-MS Ion Trap Mass Spectrometer equipped with an ESI source
- Varian ProStar™ 430 AutoSampler

Materials and Reagents

Optima LC/MS grade acetonitrile was purchased from Fisher Scientific (Part Number A955-4).

Sample Preparation

A vulcanized rubber direct extract was received in 2 mL of dichloromethane. This extract was diluted 1:10 in acetonitrile and transferred to a 2-mL amber glass autosampler vial.

HPLC Conditions

Column:	Pursuit™ XRs C18, 3 µm, 150 x 3.0 mm (Varian Part Number A6001150X030)
Mobile Phase:	Acetonitrile
LC Program:	Isocratic flow at 200 µL/min
LC Run Time::	30 min
Injection Volume:	20 µL

MS Conditions

Ionization Mode:	ESI Positive
Nebulizing Gas:	35 psi
Drying Gas:	10 psi at 350 °C
Needle:	5000 V
Capillary:	80 V
Shield:	600 V
RF Loading:	100%
Scan Parameters:	Full Scan *m/z* 100-1000
Detector:	1900 V

TurboDDS Scan Functions

Survey Scan:	*m/z* 100-700
RF Loading:	100%

MS Survey Scan Trigger Conditions

Threshold:	8600 counts
Minimum Intensity Ratio:	1%
Isotopic Equivalence:	3 *m/z*

Results and Discussion

The analysis of the vulcanized rubber formulation extract involved three steps. The first step was a screening acquisition in which the mass spectrometer was set up to perform a full scan analysis over the expected mass range of the components. This allowed for the identification of prominent protonated molecular ion peaks and the identification of polymers that are expected as a result of the vulcanization process. The second step of the analysis was the use of TurboDDS™ software to

automatically find prominent chromatographic peaks and trigger the collection of MS/MS data for further structural analysis. The third and final step of the analysis utilized ACD/MS Manager software to confirm the location in the chromatograms of certain suspected chemical components in the extracts. The ACD/MS Manager software allows the user to draw the chemical structure, attach it to the suspected MS/MS spectrum of the compound of interest, and confirm its identity by generating a list of predicted fragments and matching them to the masses found in the spectrum. The software then generates a score to indicate how well the spectrum matches the proposed fragment list.

Part I: Full Scan Analysis

First, the extract was analyzed in full scan mode. After surveying the full scan chromatogram, peaks of interest were found and extracted to yield the extracted ion chromatogram (EIC) seen in Figure 2.

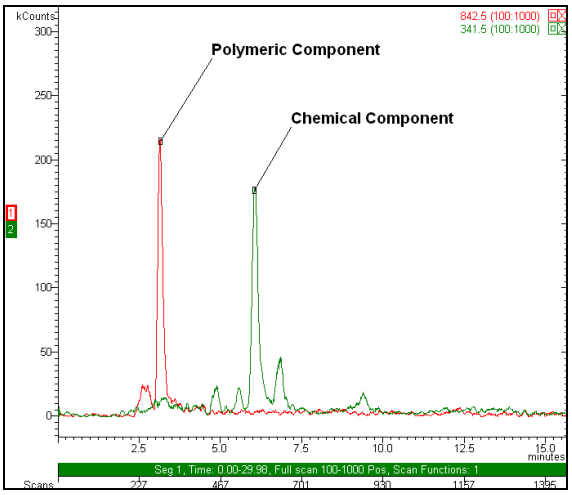

Figure 2. EIC for polymeric and chemical components of interest in the extract.

The polymeric component of the extract was identified by the characteristic cluster of evenly spaced mass spectral peaks. In this case, the 44 *m/z* spacing corresponds to C_2H_4O units (see Figure 3). The suspected chemical component of the extract (see structure in Figure 4) has an *m/z* value of 341 for the protonated molecule, which matches the mass spectrum observed for the extract.

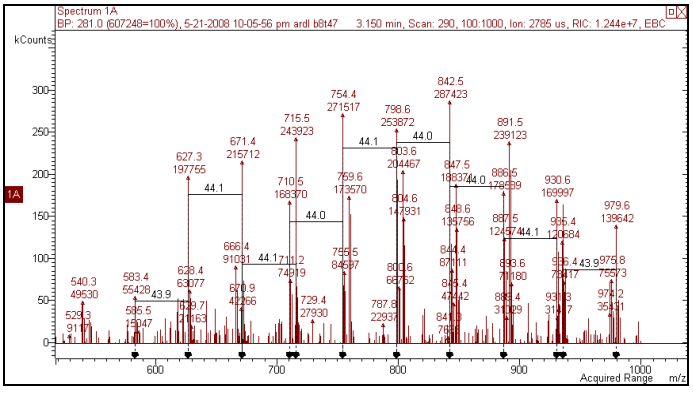

Figure 3. Polymeric component of the extract. Mass spectral peaks are evenly spaced 44 *m/z* units apart.

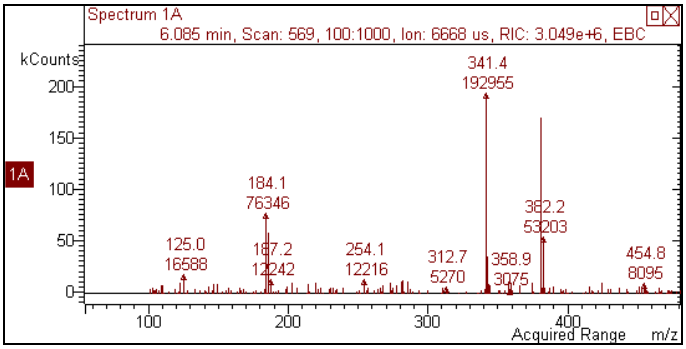

Figure 4. Full scan mass spectrum of the chemical component, suspected to be Antioxidant 2246. A protonated molecule appears in the spectrum at 341.4 u.

Part II: Structural Analysis of Chemical Component Using TurboDDS Software

The extract was shown by full scan analysis to contain a polymeric component with 44 u monomeric units, and a chemical component with *m/z* 341 (Figures 3 and 4). The next step was to analyze the chemical component using TurboDDS so that MS/MS data could be submitted for structural confirmation (Figure 5).

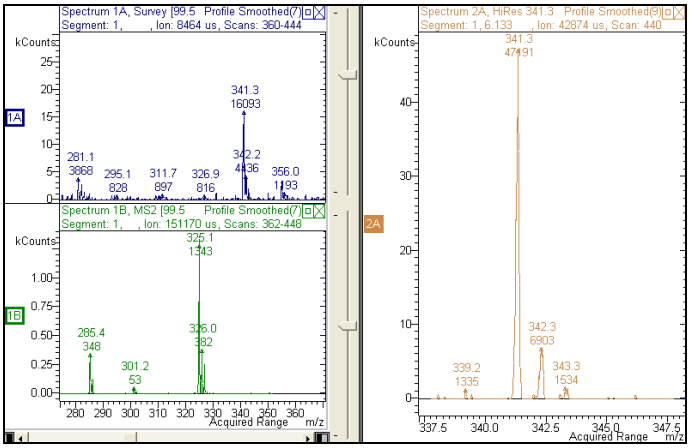

Figure 5. TurboDDS spectrum for the extract. Top left: survey scan spectrum for *m/z* 341. Bottom left: MS/MS spectrum for *m/z* 341. Right: high-resolution spectrum for *m/z* 341 precursor.

The TurboDDS analysis of the extract did trigger MS/MS analysis of *m/z* 341. The most abundant product ion from the fragmentation of *m/z* 341 was *m/z* 325. This MS/MS data was then used for structural analysis and confirmation (see Part III).

Part III: Structural Identification and Confirmation Using ACD/MS Manager Software

For confirmation of the chemical component in the extract, the structure for Antioxidant 2246 was drawn using Chem Sketch and attached to the MS/MS spectrum from the TurboDDS analysis in the ACD/MS Manager Software. Predicted fragments were matched to the acquired data using ACD/MS Processor, a component of the ACD/MS Manager software. Figure 6 shows the MS/MS spectrum for the extract with Antioxidant 2246 structure attached. The predicted fragments from the list are colored in red on the spectrum, and 68.3% of the spectral peaks were explained by the predicted fragments.

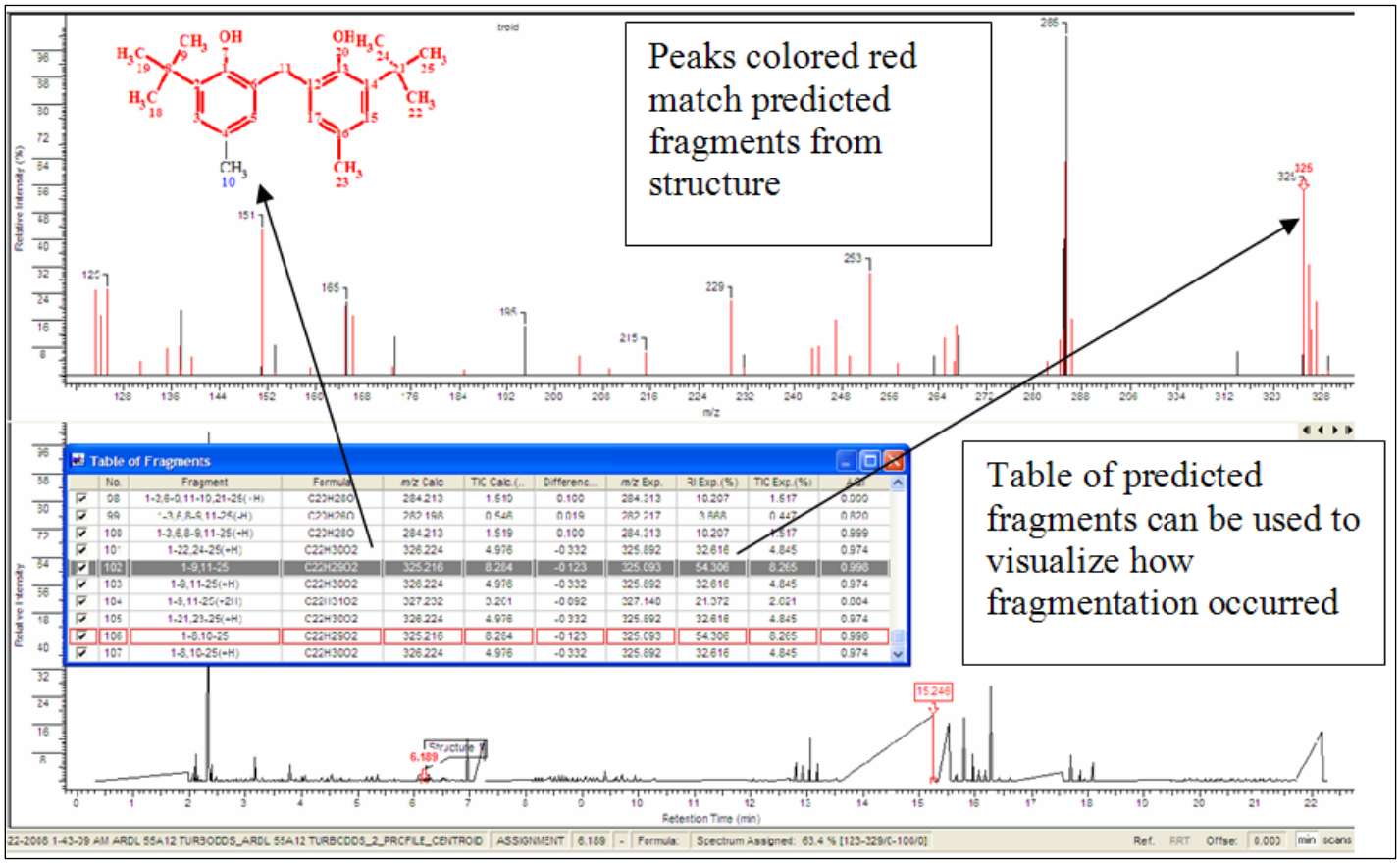

Figure 6. ACD/MS Processor overlays predicted peak matches with MS/MS data generated by TurboDDS™ analysis of the extract.

Conclusion

The MS data generated from the simple extract of vulcanized rubber formulations reveals many similarities with respect to polymers synthesized and expected components. The 500-MS with the TurboDDS™ software is very easy to use as a "walk-up" system. It allows the analyst to screen all components in a complex mixture and quickly organize all of the MS/MS data for structural elucidation. The MS/MS data generated from the peaks that the software finds is easily transferred to the ACD/MS Manager software to confirm the structure. The presence of a common antioxidant (AO 2246) used in the industrial process was easily confirmed with a high degree of confidence. This structural elucidation and confirmation technique can be applied to any application in which the components must be screened to determine contamination of a process or to identify new desirable components in a process.

These data represent typical results.
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