Screening and Qualitative Identification of Antioxidant Polymer Additives by HPLC with UV/VIS and APCI-MS Detection

Application

Consumer Products

Author

Michael Woodman
Agilent Chemical Analysis Solutions
Wilmington, DE
USA

Abstract

Liquid chromatography with ultraviolet/visible spectroscopy and mass selective detection is a powerful approach to antioxidant analysis and identification. Examples illustrate that mobile-phase conditions affect the quality and usability of the acquired data. Unknown compounds can be identified with sufficient MS data and additive degradation can be quickly evaluated.

Introduction

Plastic products are an essential part of our lives today. Whether they are used for automotive components, CDs, toys, or biocompatible replacement parts for humans, they are the subjects of intense research into new and improved polymers and blends. Equally important is the selection and quantity of chemical additives which are used to provide color, density, opacity, stiffness, flexibility, resistance to heat, light and air, flame retardance, and to improve processing properties during pellet creation and final product fabrication.

This application note examines several antioxidant (AO) types, their chemical composition, and suitable high-performance liquid chromatography (HPLC) conditions for assessing their concentration and identity, as well as their degradation products.

AOs arise from various compound classes including small hindered phenols, large hydrophobic hindered phenols, and phosphite or phosphonate linked aromatics. Examples appear in Tables 1 and 2.

Table 1. AO Studied with Structures

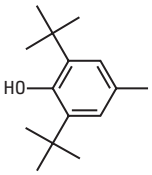
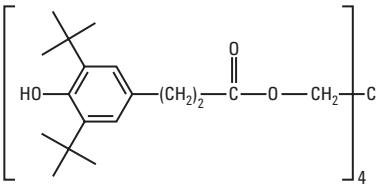
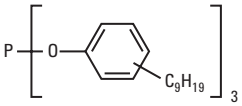
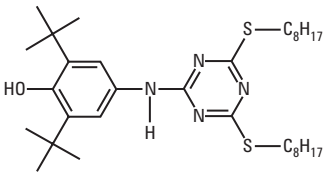
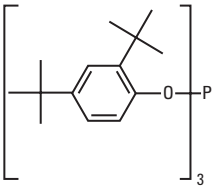
Name:	BHT	Butylated hydroxytoluene
Formula:	$C_{15}H_{24}O$	
Molecular Weight: (MW)	220.2	
Trade name:	Irganox 1010 (CibaGeigy)	Pentaerythritol tetrakis(3-(3,5-di-tert- butyl-4-hydroxyphenyl) propionate)
Formula:	$C_{73}H_{108}O_{12}$	
Molecular Weight: (MW)	1176.8	
Trade name:	Naugard P (Uniroyal)	Tris nonylphenyl phosphite
Formula:	$(C_{15}H_{23}O)_3P$	
Molecular Weight: (MW)	688.5	
Trade name:	Irganox 565 (CibaGeigy)	
Formula:	$C_{33}H_{56}N_4OS_2$	
Molecular Weight: (MW)	588.4	
Trade name:	Irgafos 168	
Formula:	$C_{42}H_{63}O_3P$	
Molecular Weight: (MW)	646.5	

Table 2. Other Common AOs

Name	Formula	MW
BHA	$C_{11}H_{16}O_2$	180.1
t-BHQ	$C_{10}H_{14}O_2$	166.1
Cyanox 1790	$C_{42}H_{57}N_3O_6$	699.4
Ethanox 330	$C_{54}H_{76}O_3$	772.6
Irganox 1076	$C_{35}H_{62}O_3$	530.5
Sandostab P-EPQ	$C_{68}H_{92}O_4P_2$	1034.6

Gas chromatographs with conventional detectors or mass spectrometers (MS) can readily analyze many small molecules; however, the increased molecular weight (MW) and decreased volatility of many AOs makes gas chromatography (GC) generally unsuitable. Liquid chromatography (LC) is a common choice because it can analyze materials exhibiting a wide MW range and varied solubility. Since LC is generally a nondestructive technique, it offers the possibility of compound isolation and recovery.

Many AOs contain functionalized aromatic groups and offer distinctive ultraviolet/visible spectroscopy (UV/VIS) spectral opportunities. This detector type is an essential part of an additive analysis system. Since UV/VIS detectors are relatively insensitive to the chromatographic mobile phase, they are readily compatible with gradient-elution separation methods.

The presence of functionalized aromatic rings, oxygen, nitrogen, phosphorous, and sulfur in many of the AOs also makes them ideal candidates for investigation by atmospheric pressure ionization mass spectrometry (API-MS). Compound identity can be supported by matching retention data, UV/VIS spectra, and from the MS, a molecular ion (essentially giving the molecular weight of the compound). Depending on the type of ionization and MS chosen, further identification can be made where higher energy is employed, causing fragmentation of the molecules. These fragments help experienced users propose chemical structures.

Instrumentation and General Method

Agilent 1100 LC system:

- Quaternary gradient pump with low volume degasser
- Binary gradient pump with degasser, for pre-MSD reagent addition
- ALS automatic sampler with 2-mL vial tray
- Thermostatted column compartment with automated 6-port, 2-position switching valve
- Diode array UV/VIS spectrophotometer

General chromatographic conditions:

- Gradient elution of increasing organic-solvent strength with combinations of:

  Water/Acetonitrile (ACN)
  Water/Methanol (MeOH)
  Water/Methanol/Tetrahydrofuran (THF), HPLC grade

- UV/VIS spectral-data collection from 200–400 nm, 1-nm slit, 4 nm resolution
- UV/VIS single-wavelength collection for 210 and 280 nm, at 4 nm resolution

ChemStation PC Data and Control System

Mass selective detector (MSD) SL single quadrupole MS with APCI interface

Fragmentor: 100 V, positive and negative ionization

Vaporizer: 400 °C

Nebulizer: 50 psi nitrogen

Drying gas: 6 LPM Nitrogen

Column: Zorbax XDB-C8, 4.6 mm id × 50 mm L, 3.5 µm particles

Gradients:

Method 1. "MeOH/THF", Column 30 °C, 25 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	50	0	10
1	15	0	90	0	10
1	20	0	90	0	10
1	21	40	50	0	10

Method 2. "MeOH", Column 40 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	60	0	0
1	10	0	100	0	0
1	15	0	100	0	0
1	16	40	60	0	0

Method 3. "ACN", column 50 °C, 20 min cycle

Flow	Time	% Water	% MeOH	% ACN	% THF
1	0	40	0	60	0
1	10	0	0	100	0
1	15	0	0	100	0
1	16	40	0	60	0

Experimental Results

Figures 1 through 3 are overlaid UV chromatograms for nine AOs, using three different gradients.

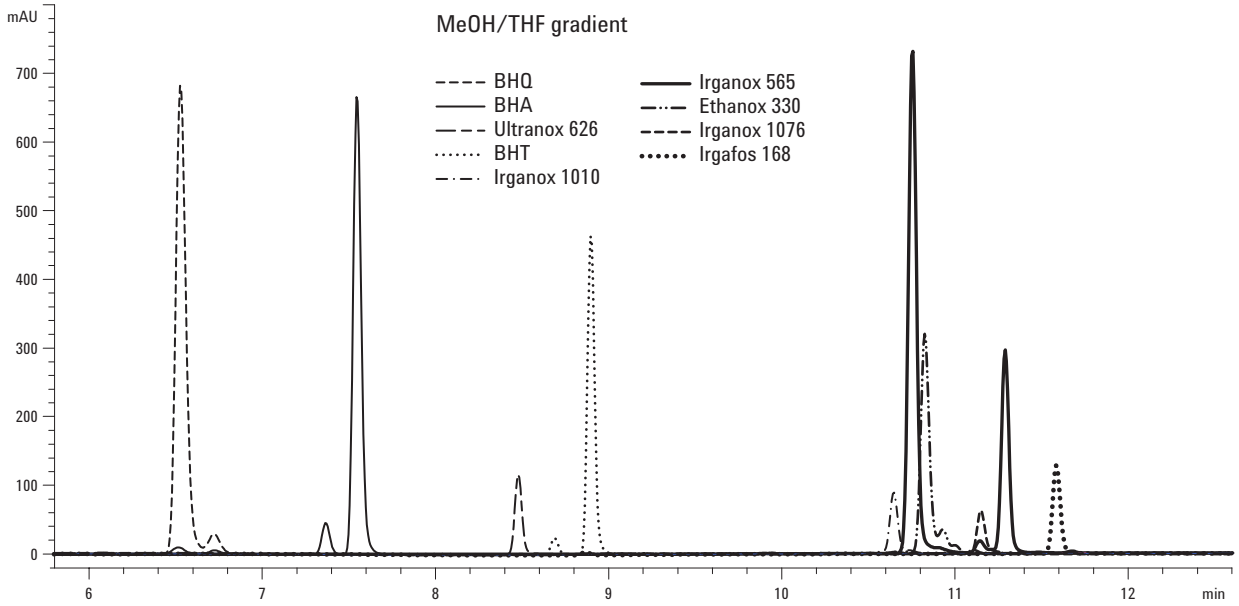


Figure 1. Overlaid UV chromatograms for the selected AOs using the methanol/THF gradient.

Many samples have minor peaks originating from impurities or degradation products having structures similar to the parent molecules. For the smaller molecules like BHA, BHQ, and BHT, there is no problem with resolution. For larger molecules, there is reduced resolution in the 10- to 12-minute region. These molecules have unique MWs, though, and can be analyzed using selective MS detection.

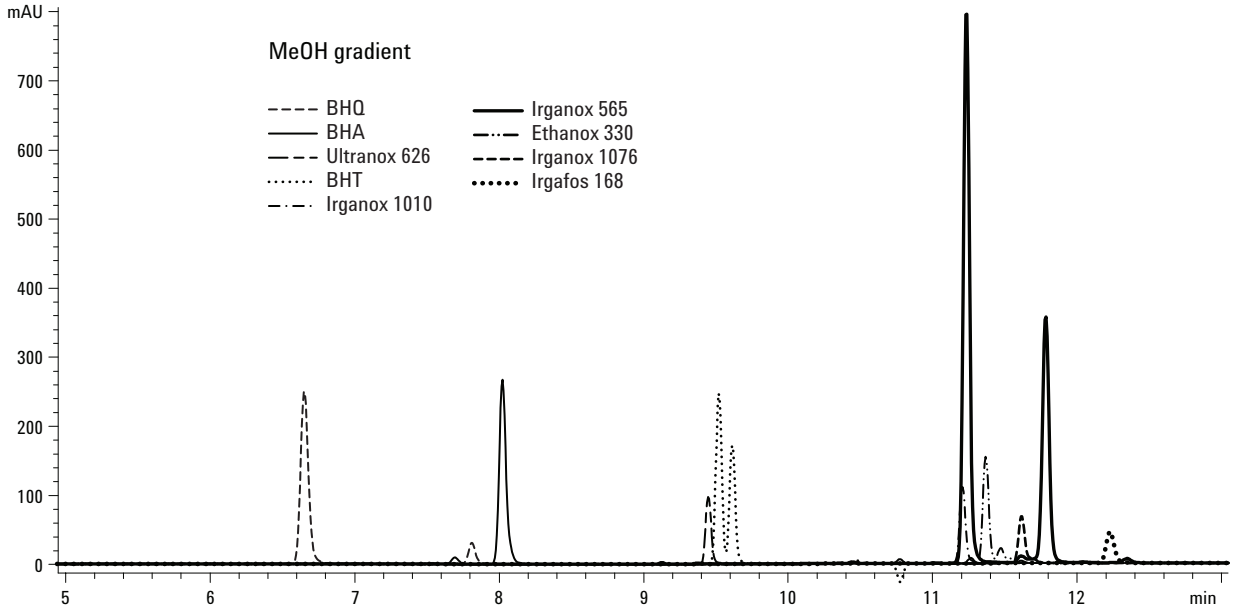


Figure 2. Separation of AOs using the MeOH gradient.

Using the MeOH gradient, relative separation is somewhat different, and as before, the smaller molecules are well resolved. The larger molecules in the 11- to12-minute region exhibit reduced resolution, but can be analyzed using selective MS detection.

Figure 3 shows the separation of the same AOs using the ACN gradient.

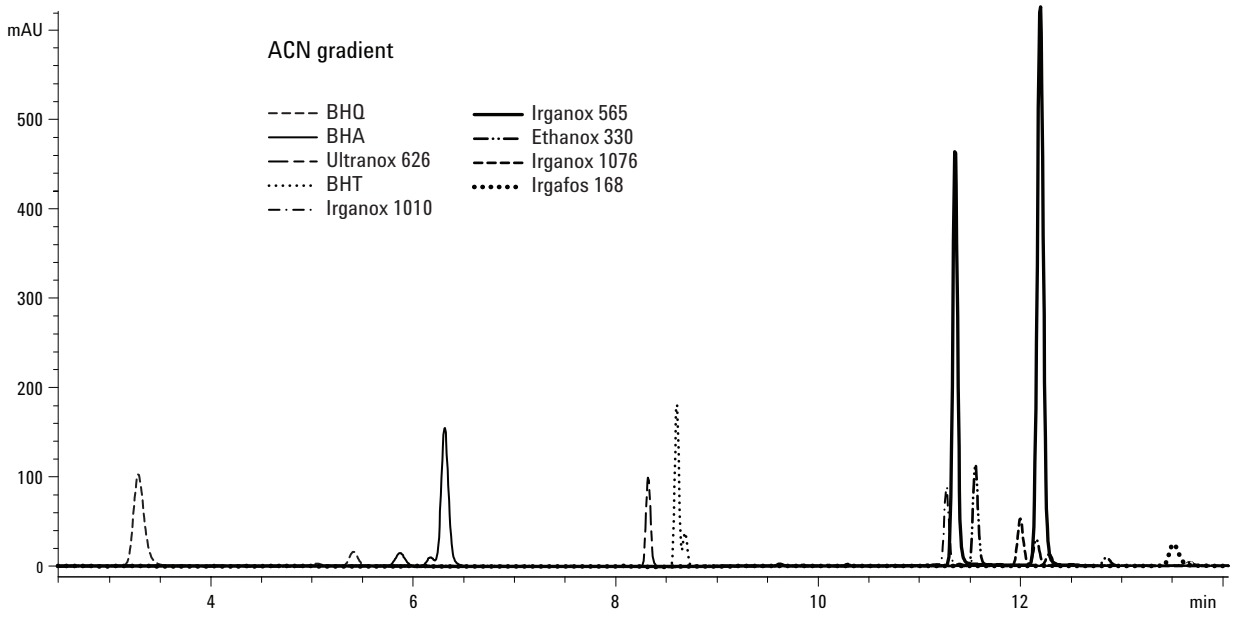


Figure 3. Separation of AOs using the ACN gradient.

Once again, no problem exists with resolution of the smaller molecules. For larger molecules in the 11- to 12-minute region there is somewhat better resolution. ACN has the best UV transparency at low wavelengths, maximizing baseline stability in the wavelength range where UV response would be observed for the AOs.

It is often attractive to use UV/VIS libraries to tentatively identify components in the sample mixture. This approach is especially useful when the various analytes have distinct spectra. Where many AOs have phenolic rings with characteristic UV/VIS spectra, distinguishing analytes by this approach is difficult and the user must rely on retention time data to support any identification attempt.

As we investigate various AO molecules, it is useful to note the general mass range for single- and multiple-ring structures. See Figure 4.

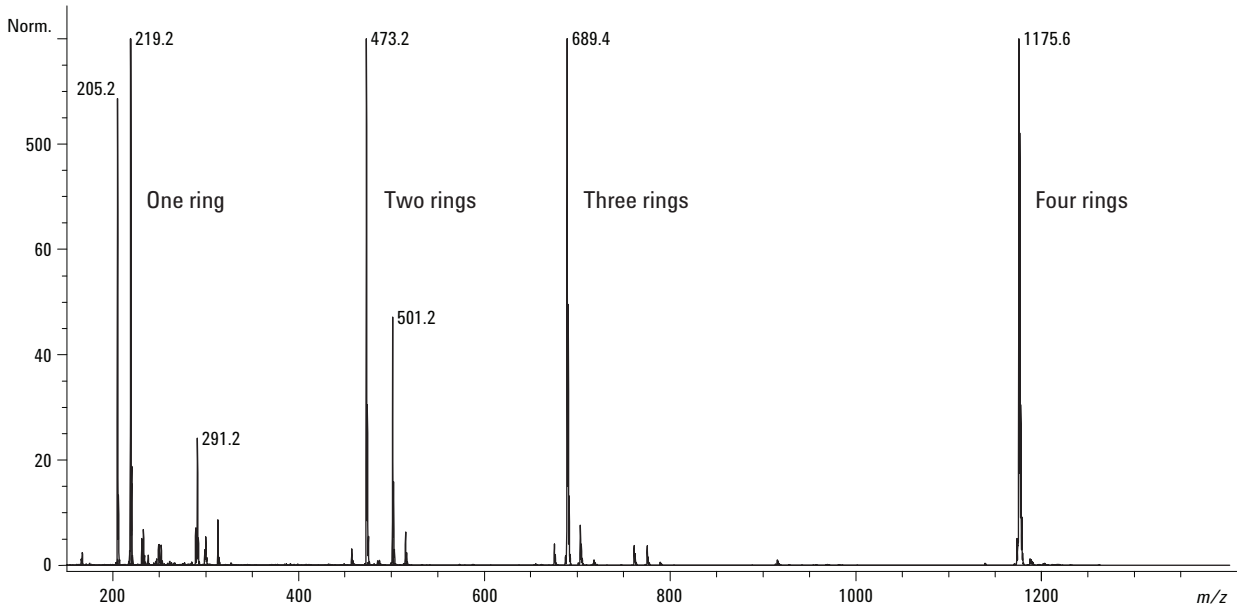


Figure 4. Overlaid AO mass spectra, illustrating effect of ring number on observed mass range.

In Figure 4 we see intact and fragmentation ions representing structures from one to four aromatic rings. The *m/z* 219 is $[M-H]^-$ for BHT while *m/z* 205, less one CH_2, is a fragmentation ion of a larger molecule having the hindered phenolic feature. The *m/z* 473 and *m/z* 501 are fragments discussed later in this text. The *m/z* 689 is Naugard P, $(C_{15}H_{23}O)_3P$. The *m/z* 1176, Irganox 1010, $(C_{73}H_{108}O_{12})$ has four rings and long alkyl chains that increase the mass and remind us that it is important to acquire mass data well over 1000 Da for general AO screening and analysis.

The mobile phase absorbance background invariably affects UV/VIS spectra. See Figure 5. In this example, the UV/VIS spectra for Irgafos 168 are shown for the three previously described solvent conditions.

Significant differences in response, especially in the important low UV range, are generally observed. This interference is also found with many ionic modifiers added to the mobile phase to control ionization of analytes, possibly improving the separation or enhancing ionization of the compounds in the MS.

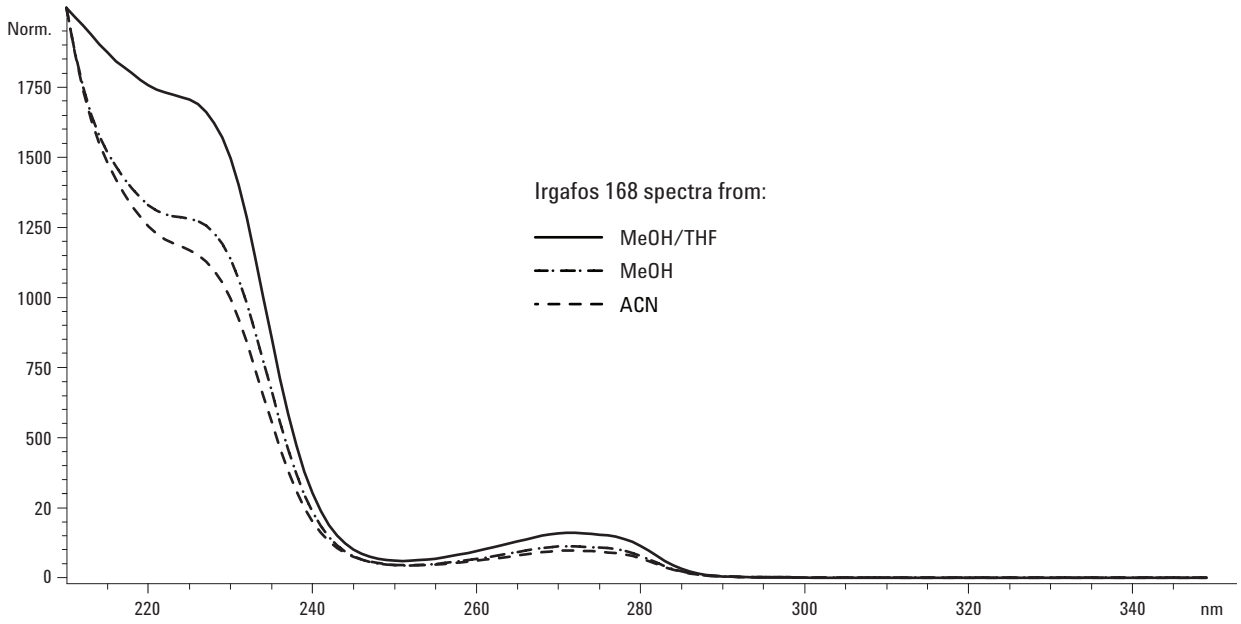


Figure 5. Solvent effects on UV/VIS spectra for Irgafos 168.

Ionization, and thus ion abundance in the MS, may also be affected by the mobile phase composition.

In Figure 6, the extracted positive-ion spectra for Irgafos 168 (molecular weight 646.5, detected as the $[M+H]^+$ ion) appear in the three previously described solvent conditions, where it elutes in high organic concentrations. Observe the significant differences in response, with the lowest response in ACN. Reduced response from the molecular ion may be from decreased ionization or increased fragmentation. It may be possible to add modifiers after the UV, and prior to the MSD inlet, to enhance MS response in circumstances where the solvent offers chromatographic or UV/VIS advantages but negatively impacts ionization in the MS.

The degree of fragmentation in the MS may also be affected by the mobile-phase composition. In Figure 7, the extracted negative-ion spectra for Irgafos 168 appear in the three previously described solvent conditions.

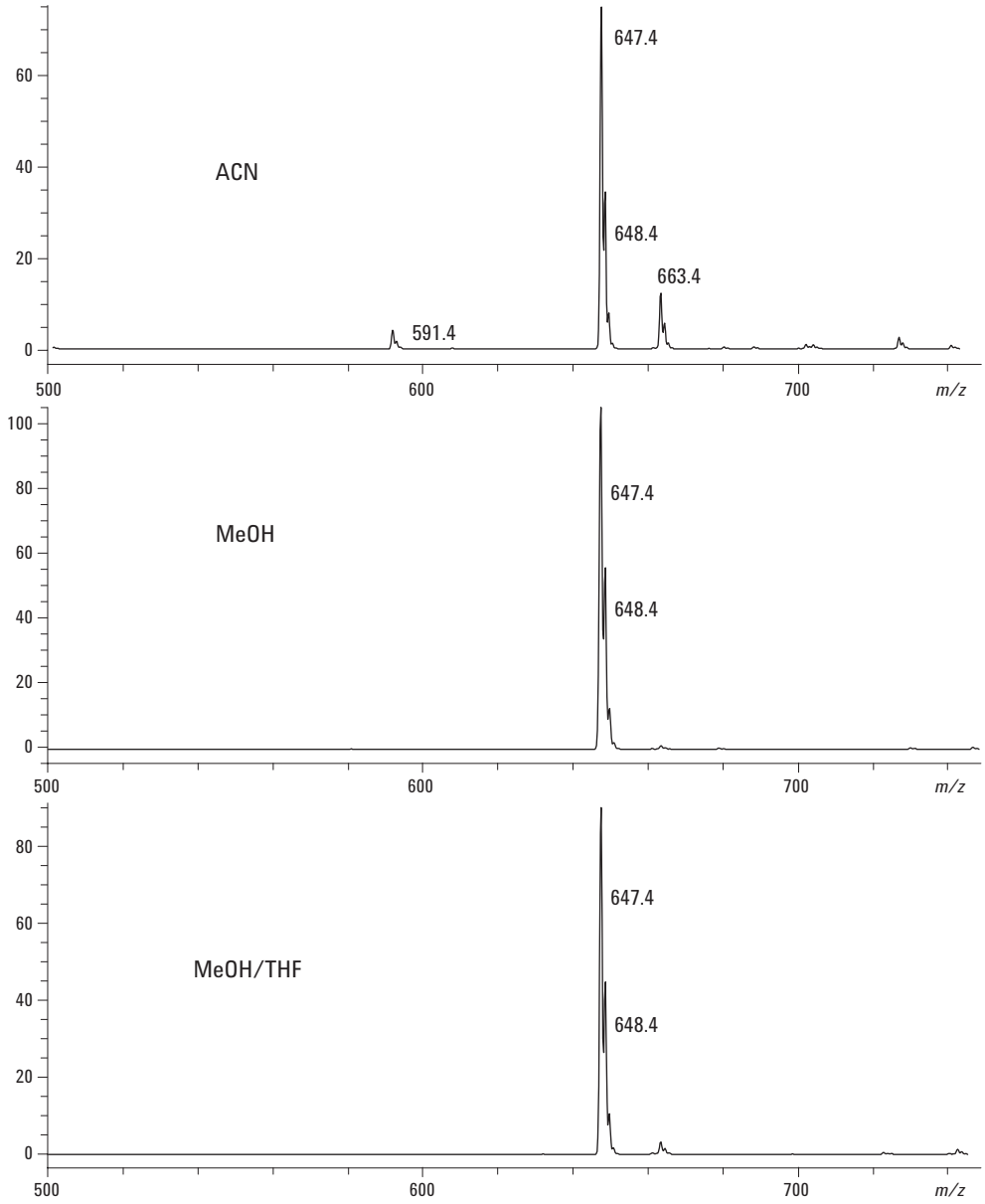


Figure 6. Solvent effects on positive-ion MSD spectra for Irgafos 168.

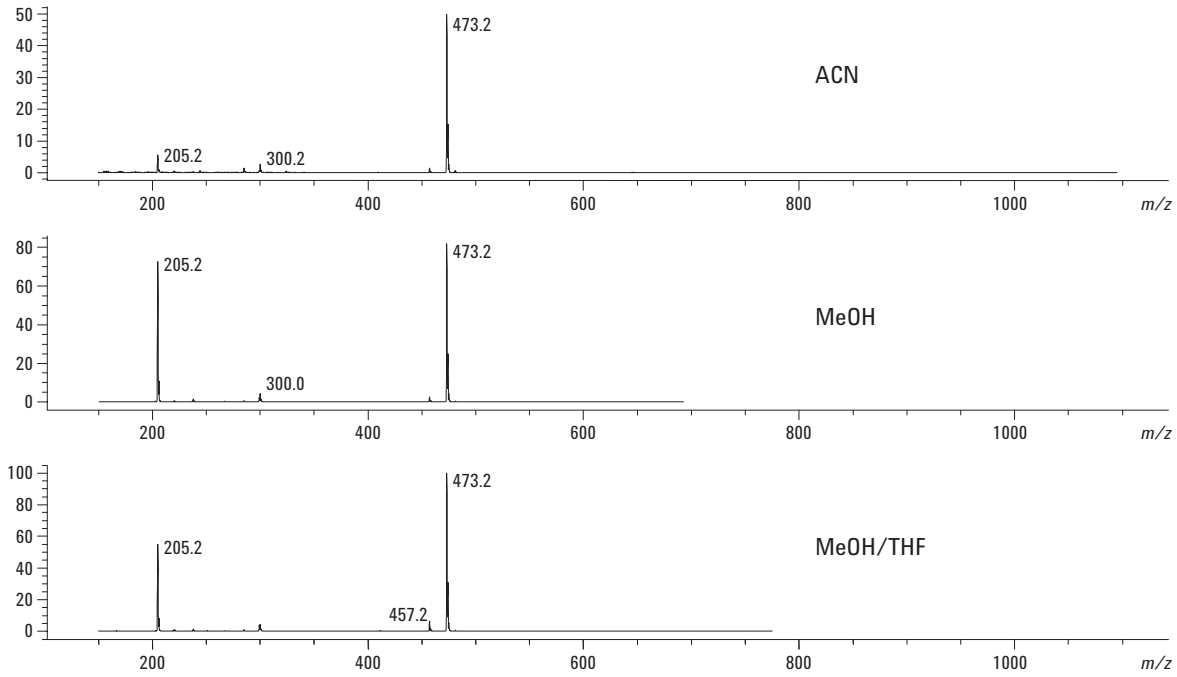


Figure 7. Solvent effects on negative-ion MSD spectra for Irgafos 168.

Note the significant differences in response with the lowest response in ACN. Reduced response for the molecular ion and fragment ions suggests that the ACN response is simply reduced ionization. Based on known degradation chemistry of Irgafos 168 and similar compounds, the *m/z* 473 fragment is likely $[C_{28}H_{42}O_4P]^-$ where an "arm" is lost (*m/z* 205) and an oxygen remains on phosphorous as P=O.

Identification of Unknowns

Retention data may allow experienced chromatographers to suggest how an unknown peak might differ structurally from a group of knowns run under the same conditions, but identification invariably takes far more resources than simple elution patterns provide. From UV/VIS data, we can often suggest molecule class, especially so in our discussion of compounds commonly having the phenoxy group in the chemical structure. UV/VIS spectra may be suggestive but, when used without significant prior knowledge, lack sufficient resolution to confirm identity. MS data, on the other hand, have the spectral resolution necessary to infer structural details leading to actual chemical identification. The following examples describe several situations in which either detector would be helpful.

In the simple case of an unknown containing either BHA or BHT, the UV spectra (Figure 8) are sufficiently unique to allow a reasonable identification along with characteristic retention data. Nearly 1.5 minutes separate these two peaks in the conditions above and little doubt would remain.

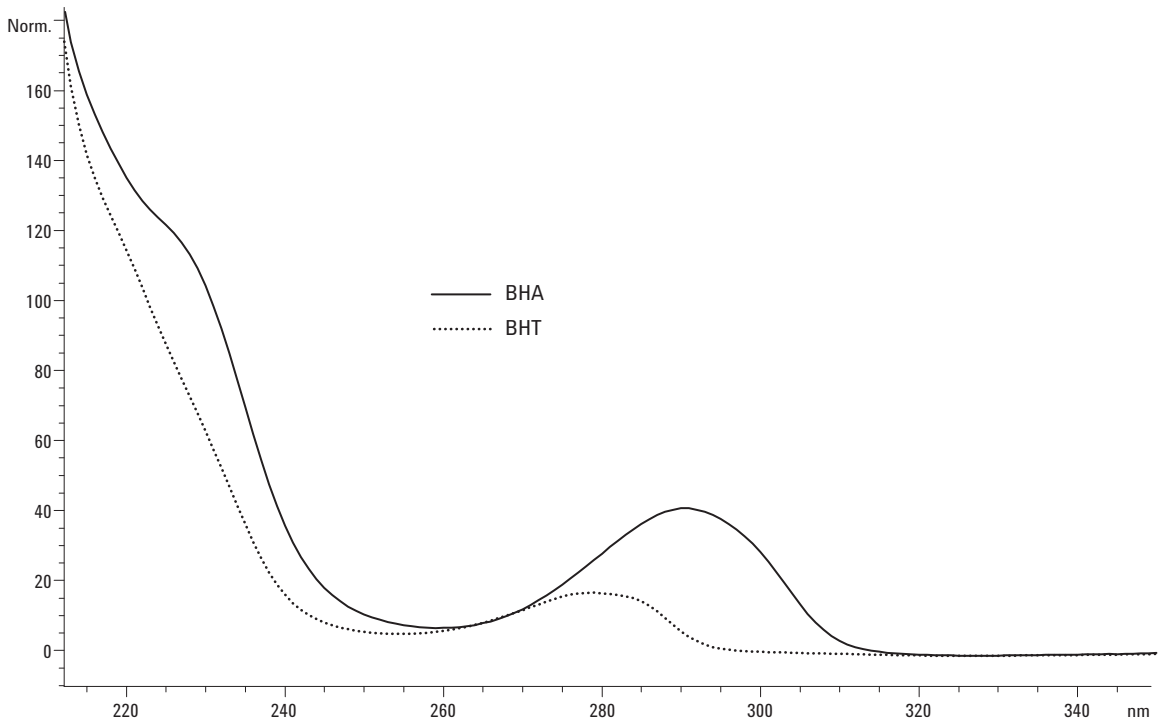


Figure 8. Extracted UV spectra from mixture containing only BHA and BHT.

Using MS data for the same sample, we would reach similar conclusions. See Figure 9.

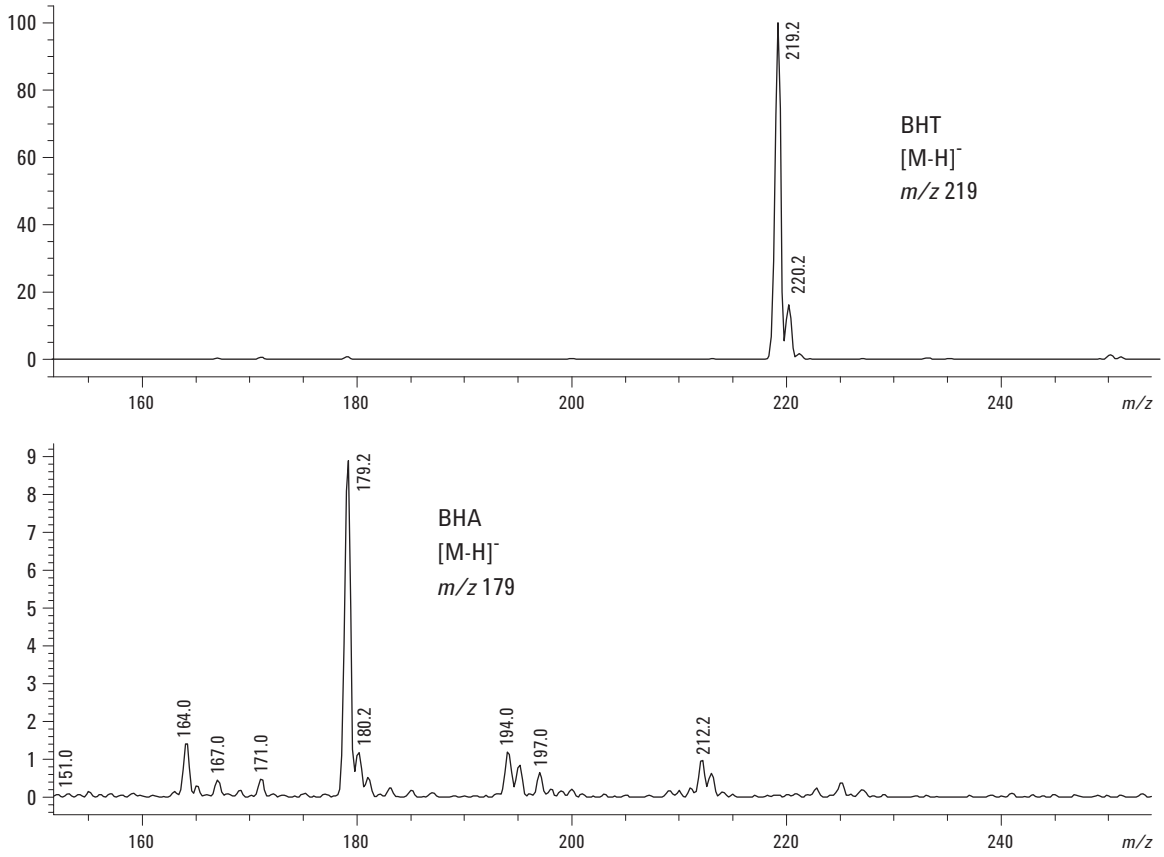


Figure 9. Extracted negative-ion MS spectra from mixture containing only BHA and BHT.

Retention data suggests two distinct molecules leading to an unambiguous identification without any need for MS fragmentation data.

When examining MS data, we generally expect to see classic molecular ions, either molecular mass+1 in positive-ion mode or mass-1 in negative-ion mode. These conditions, in the absence of significant adduct or fragment ion formation, often yield the best sensitivity and quantitative result. Such is the case in the Irganox 565 example shown in Figure 10.

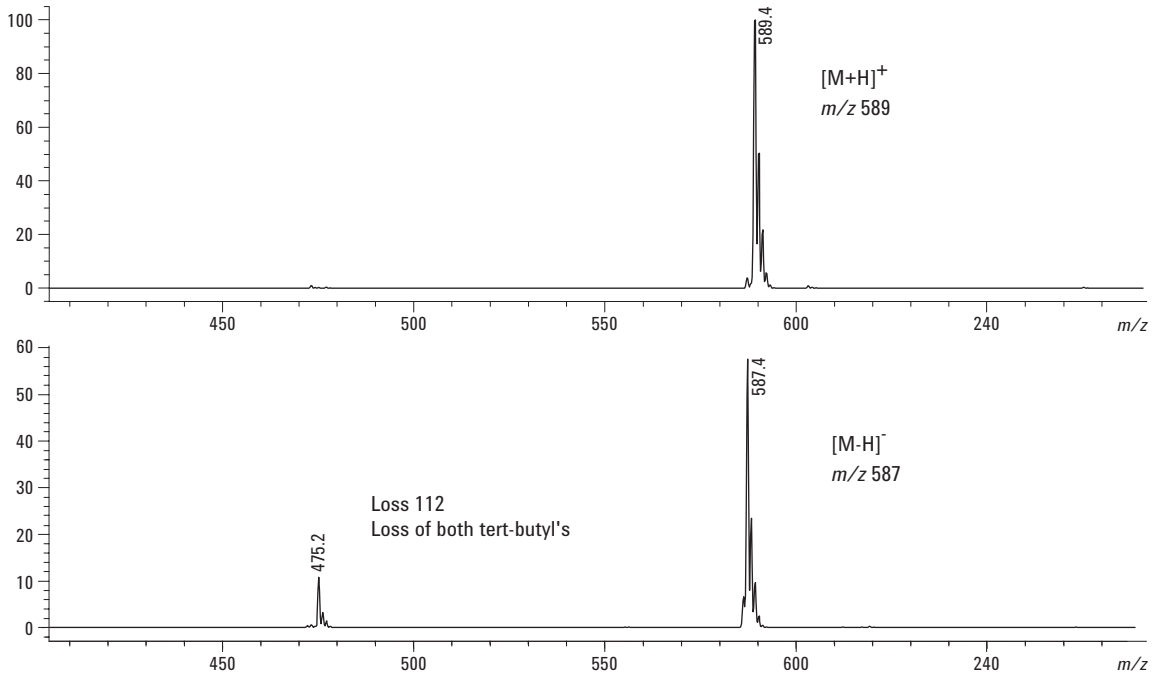


Figure 10. Positive- (upper) and negative- (lower) ion spectra for Irganox 565, using MeOH/THF gradient.

Only minor amounts of fragmentation are seen in the negative-ion spectrum, corresponding to the loss of both tert-butyl groups. In some cases, a radical ion is formed and the MS ion observed will correspond to the mass of the parent molecule. It is difficult to predict when this may occur, but the user must be prepared to interpret the spectral data with this situation in mind.

Irganox 1010 was run under the same conditions and produced minimal fragmentation in the negative-ion spectrum. An $[M\text{-}H]^-$ ion at *m/z* 1175.6 is detected for the expected MW 1176.8. See Figure 11.

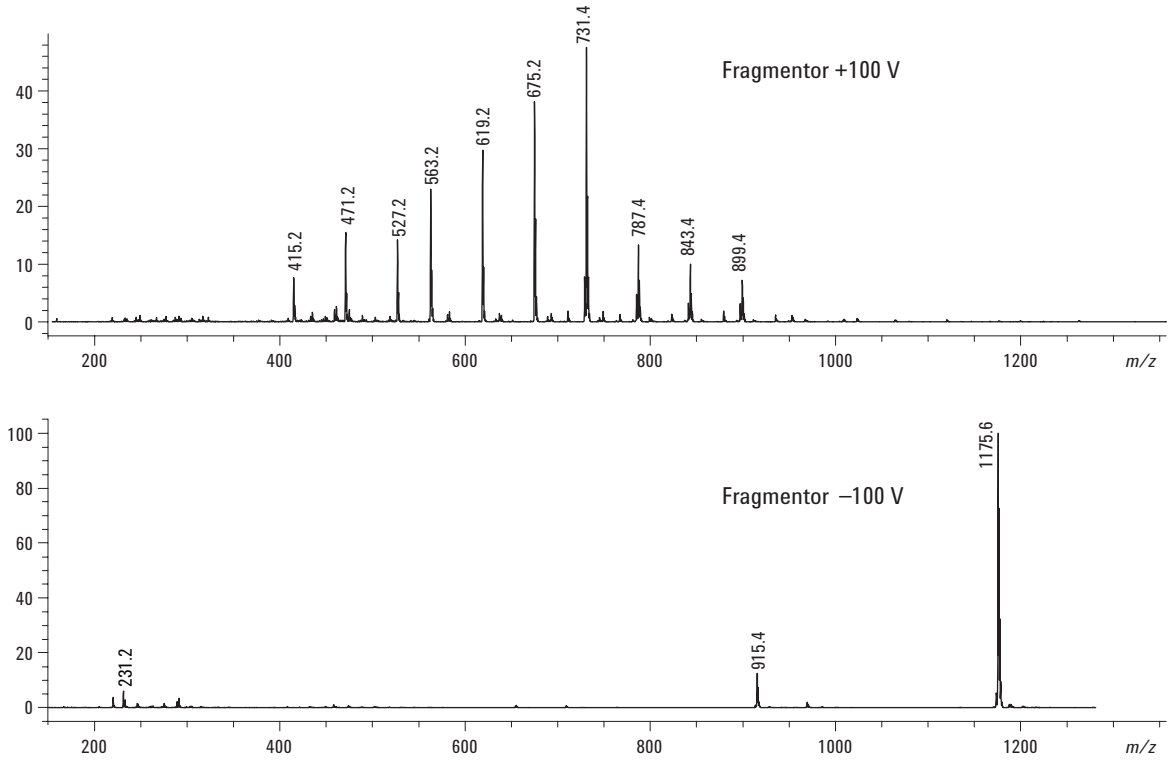


Figure 11. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH/THF gradient.

The positive-ion spectrum, however, is devoid of any useful amount of the molecular ion. The resulting fragmentation pattern suggests a molecule with a significant number of tert-butyl structures which, with the molecular ion from negative ionization, is consistent for a tentative identification for the named compound.

Little change is observed in the fragmentation pattern by reducing the fragmentor voltage to 25 V, though overall ion production is reduced from the 100 V experiments. See Figure 12.

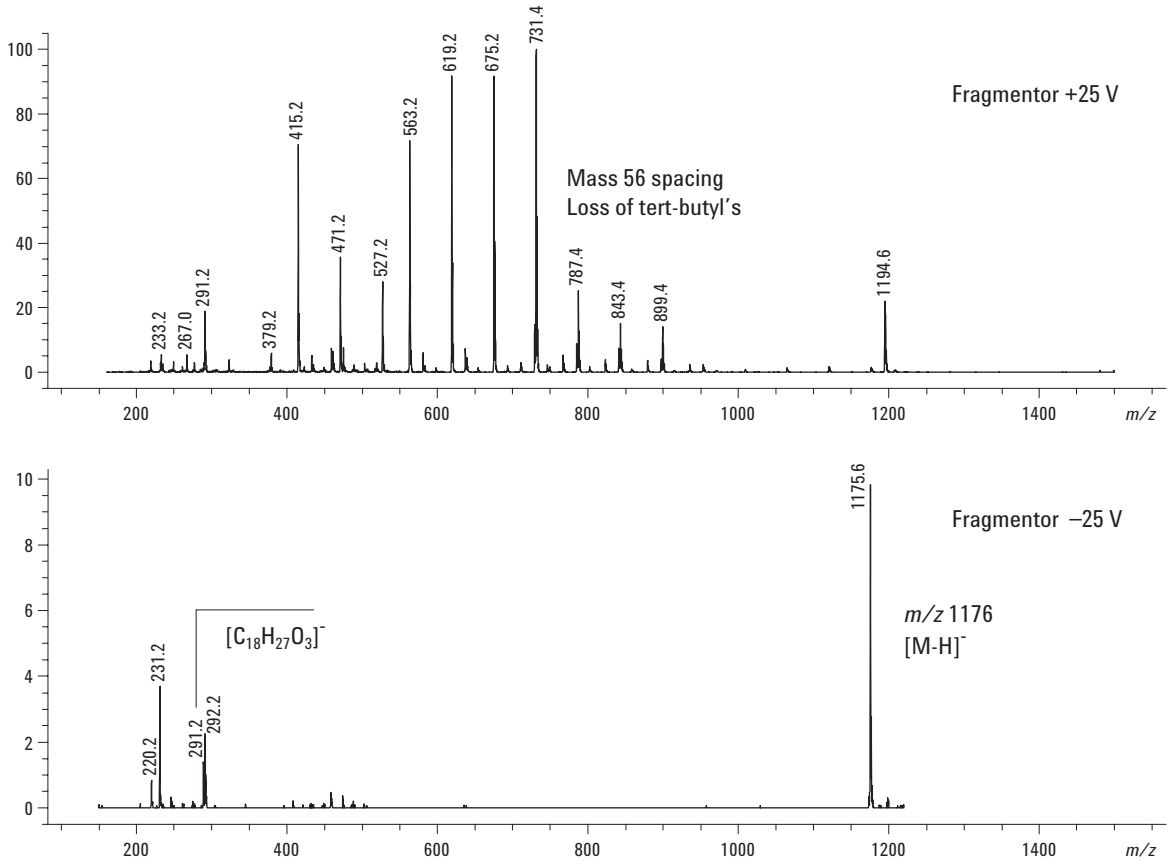


Figure 12. Positive- (upper) and negative- (lower) ion spectra for Irganox 1010, using MeOH gradient.

An *m/z* 291 fragment ion can be observed, which corresponds to one of the symmetrical "arms" of the molecule.

The positive- and negative-ion spectra extracted from the main peak in a degraded standard of Naugard P appear in Figure 13. Naugard P responds comparably to the Irganox 1010 in positive-ion mode, yielding an easily observed molecular ion.

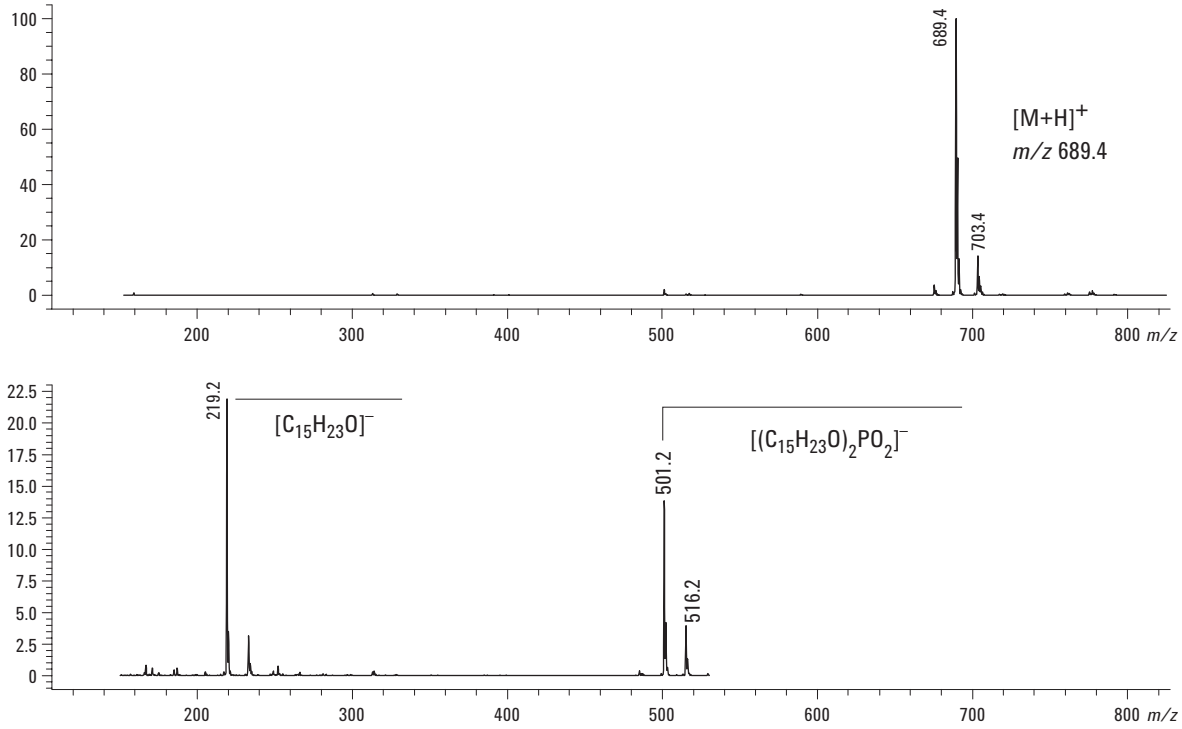


Figure 13. Extracted positive- (upper) and negative- (lower) ion spectra from the main peak in a degraded Naugard P standard.

Poor response in negative-ion mode is presumably due to excessive fragmentation, and no molecular ion is observed. Fragments and minor rearrangements found under these conditions are excellent markers for this sample type and would be good indicators if unknown samples were analyzed.

Peaks in the degraded Naugard P analysis have characteristic positive- and negative-ion spectra which could be studied to confirm typical or propose unknown degradation products. All the peaks seem to have the alkyl side chain present. The other variations presumably lie with the number of oxygen atoms attached to the phosphorous, as proposed in the spectra of the peak at 11.6 min in Figure 14.

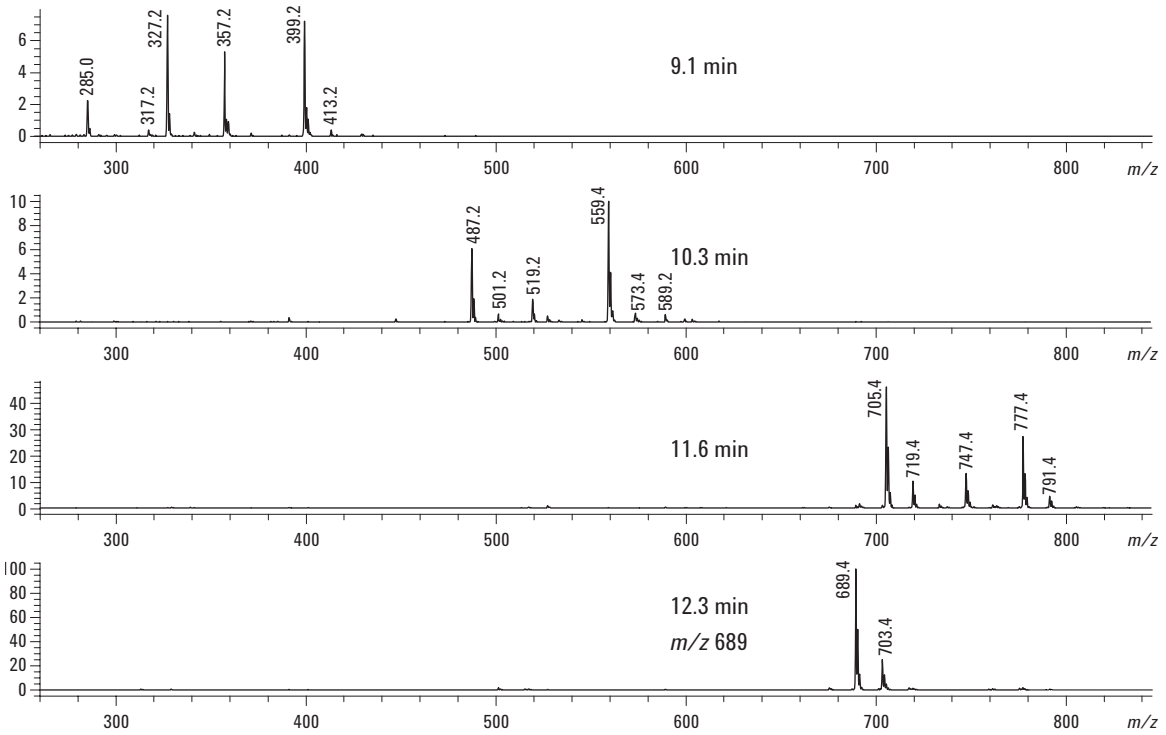


Figure 14. Extracted positive-ion spectra for Naugard P.

Likewise, the negative-ion fragmentation patterns shown in Figure 15 help simplify the investigation by showing differences in the alkyl chain or P-O bonds.

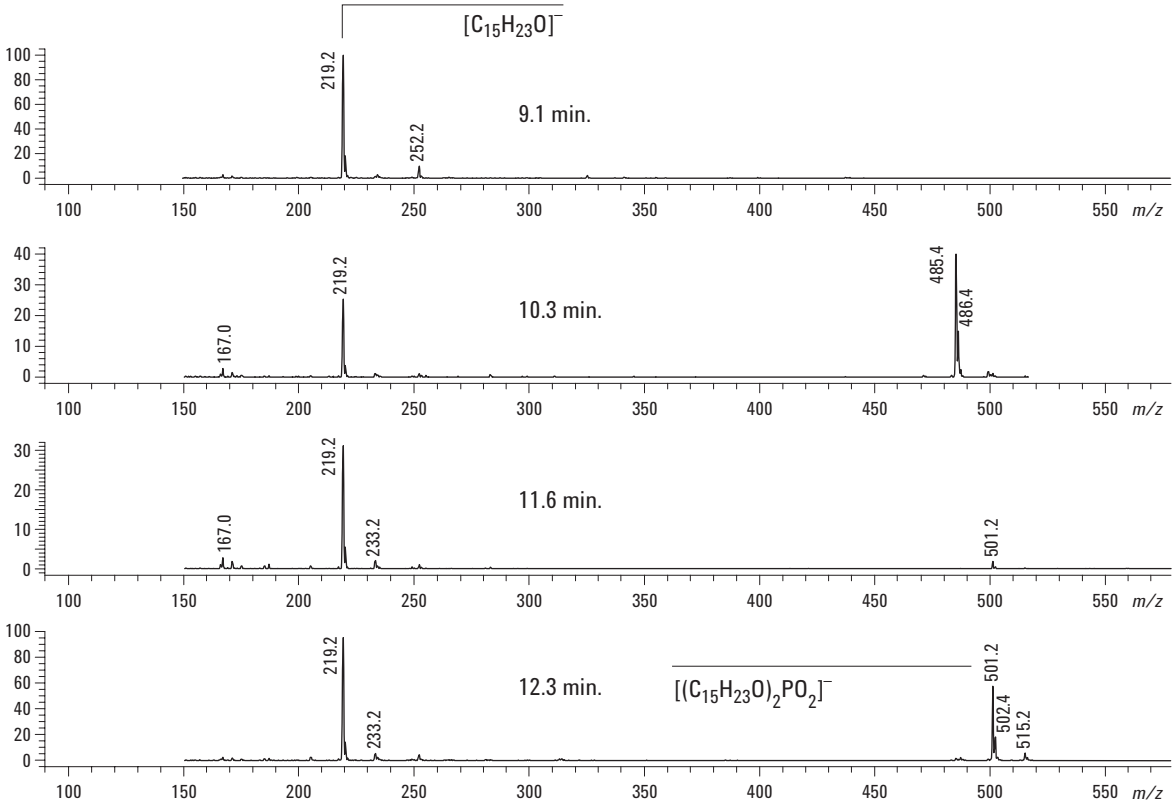


Figure 15. Extracted negative-ion spectra for Naugard P.

We received several unknown samples containing polymer additives. The prepared solutions were analyzed with a wide variety of known standards of AOs and other additive classes. Of all the analyzed standards, Naugard P chromatographic patterns, as shown in Figure 16, most closely matched the unknown samples. Additional spectral investigations followed.

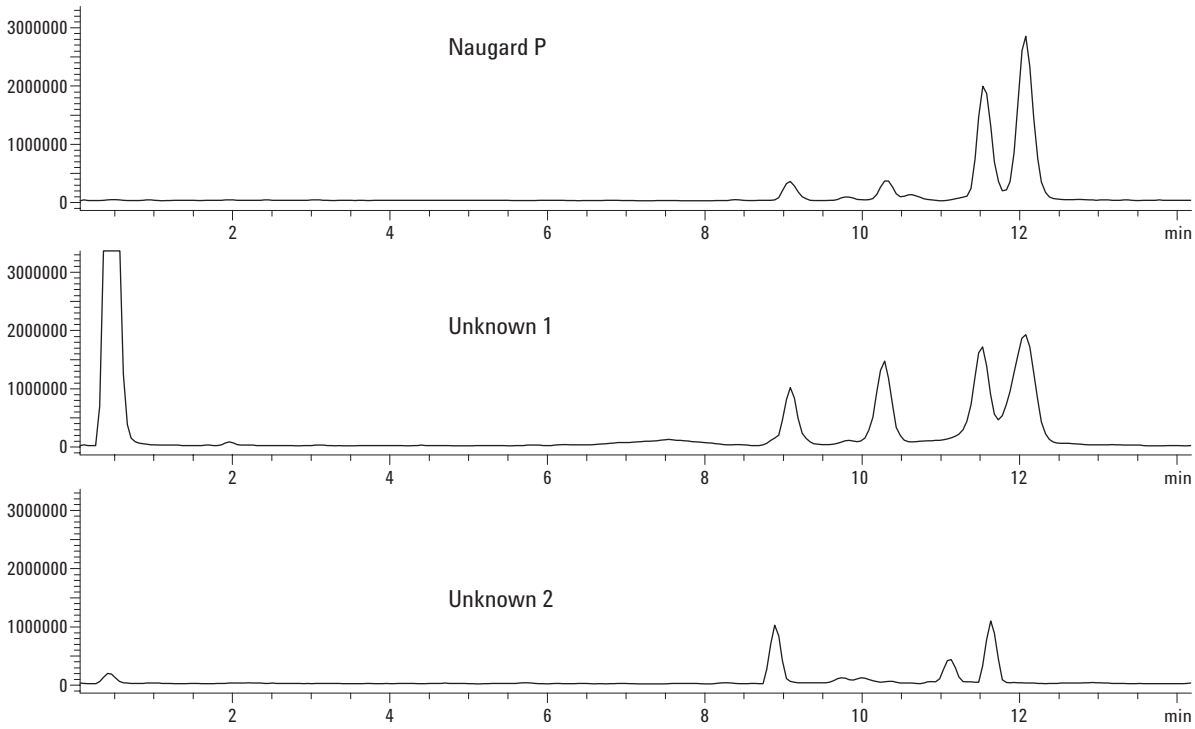


Figure 16. Total positive-ion chromatograms of Naugard P and two unknowns are compared.

The UV spectra for these same samples shown in Figure 17 are similar, though still generally characteristic of many aromatic compounds having minimal ring substitution. These data are interesting, but not conclusive.

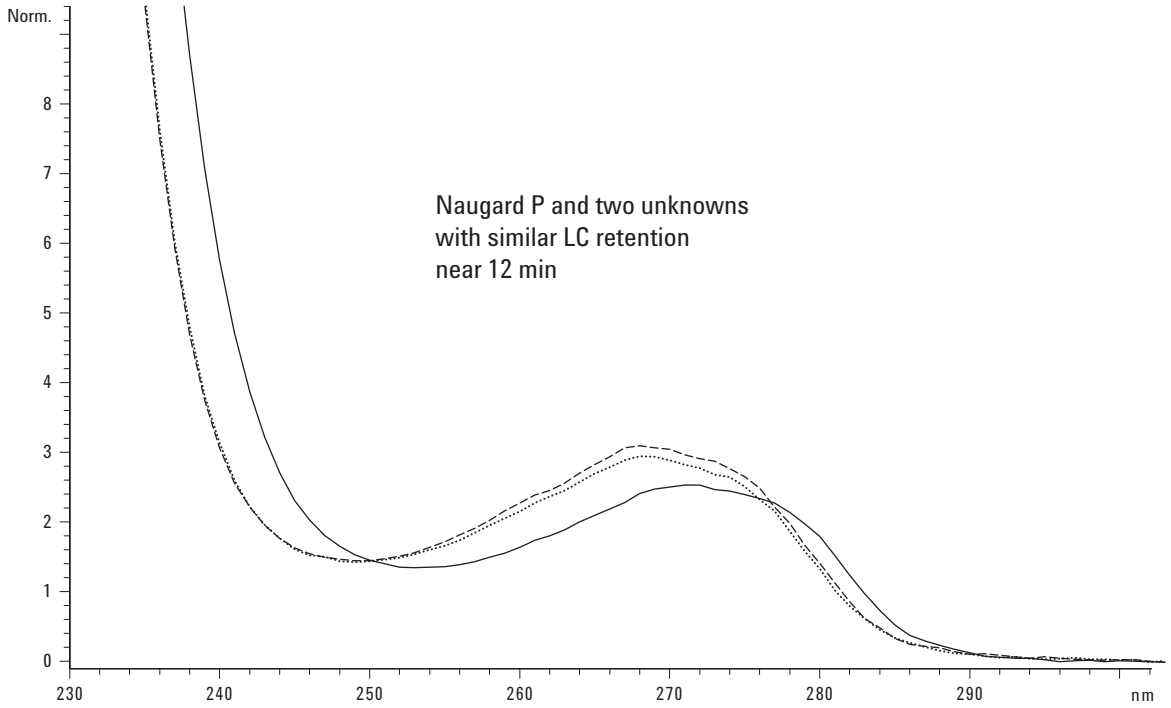


Figure 17. UV spectra of Naugard P and the two unknowns.

The positive-ion mass spectrum of Unknown 1, shown in Figure 18, is an excellent match to that of Naugard P, showing slightly more alkyl variation than the standard. This could be a different lot of Naugard P or a product from a different supplier. Unknown 2 has the primary positive-ion at *m/z* 647, reasonably due to a shorter alkyl chain, C_8H_{17}, compared to the C_9H_{19} alkyl chain on Naugard P.

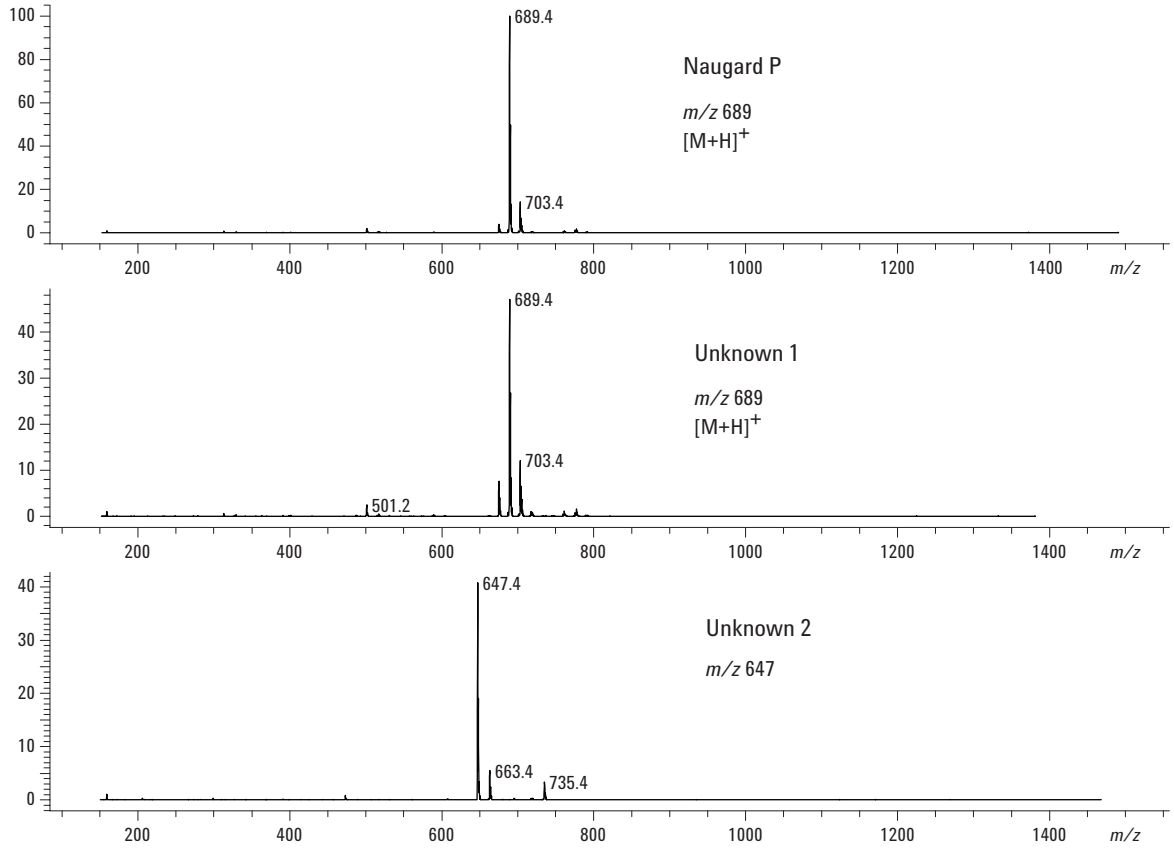


Figure 18. The positive-ion mass spectra of Naugard P and the two unknowns.

In negative-ion mass chromatograms, we see similarities to Naugard P in Unknown 1 and quite dissimilar data in Unknown 2. Recalling from earlier discussions that Naugard P is highly fragmented in negative-ion mode, the negative-ion mass spectra should be extremely helpful in supporting our initial thoughts taken from the positive-ion spectra. See Figure 19.

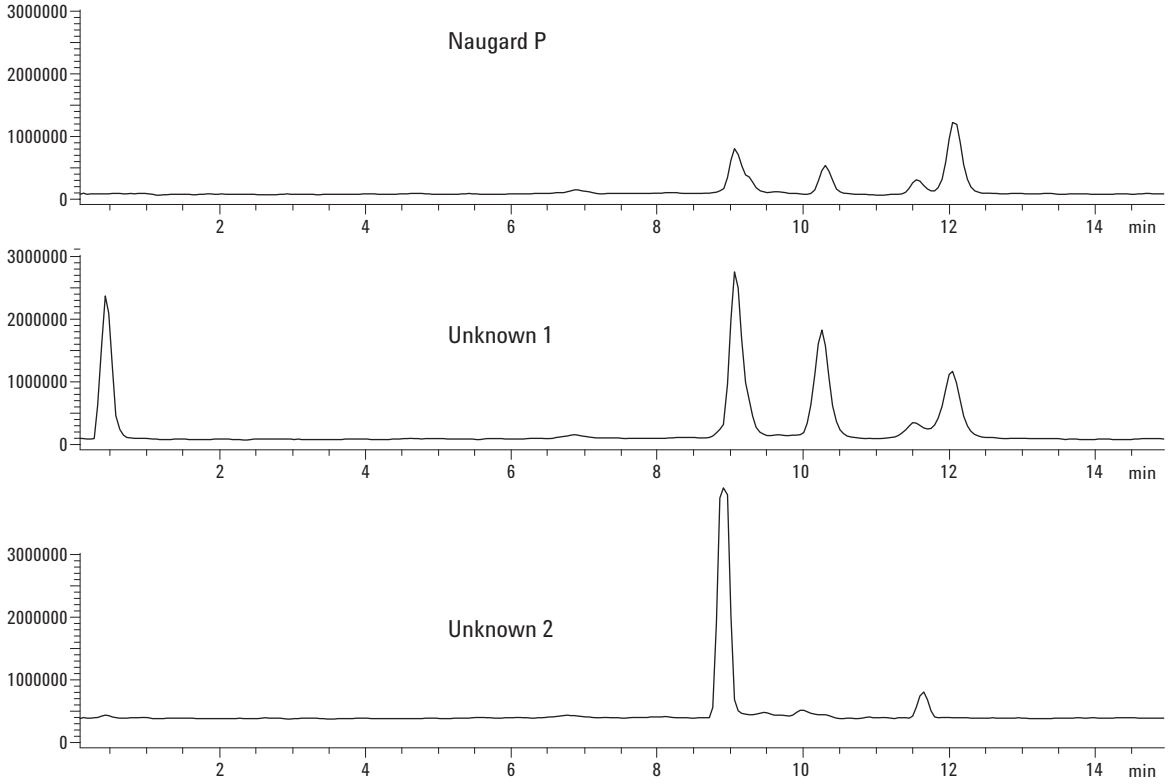


Figure 19. Total negative-ion chromatograms of Naugard P and two unknowns are compared.

The negative-ion spectra for Naugard P and Unknown 1 are an excellent match and probably offer the best support of that chemical identity and structural details. Unknown 2, however, speculatively presents two CH_2's less in the *m/z* 501 fragment and one CH_2 less the *m/z* 219 fragment. See Figure 20. This is highly supportive of the proposed structure from the positive-ion data and allows us to conclude that, while similar to Naugard P, it is a unique product whose structure is most likely $(C_6H_4\text{-}C_8H_{17}\text{-}O)_3P$.

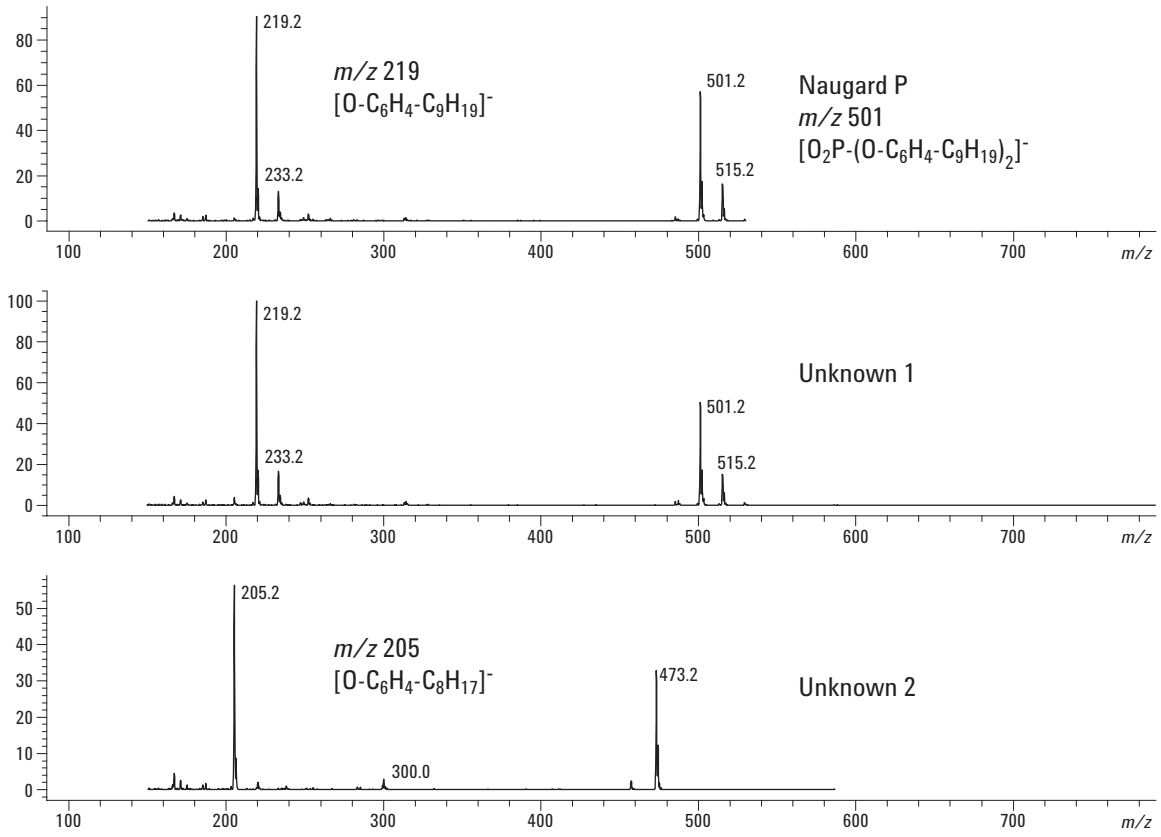


Figure 20. Negative-ion fragmentation mass spectra of Naugard P and the two unknowns.

Conclusions

- LC with UV/VIS and MSD detection is a powerful approach to compound analysis and identification.
- Mobile phase conditions affect the quality and usability of the acquired data.
- Unknown compounds can be tentatively identified with MS data.
- Additive degradation can be quickly evaluated to optimize formulations for better performance.

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Printed in the USA
March 17, 2003
5988-8610EN

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