APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Meeting EU REACH Requirements using Chemical Ionization Gas Chromatography/ Mass Spectrometry

Introduction

Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) is a European Union Regulation (EC No. 1907/2006) aimed at protecting human and environmental health along with the competitiveness of the chemical industry within the European Economic Area. A main provision of the regulation is the transfer of responsibility from central governments to the manufacturers or importers of chemicals to ensure compliance of their chemical products. This applies to substances imported in quantities above 100 tons, compounds "intended for release" from a product, or compounds classified as a Substance of Very High Concern (SVHC). The manufacturers and importers must evaluate these chemicals and manage any identified risk. Included among these requirements is the chemical analysis of these substances.

In this application note we describe how gas chromatography/mass spectrometry (GC/MS) utilizing the chemical ionization (CI) technique can help characterize chemicals for REACH registration and compliance. The positive identification of 4,4'-Methylenebis(2,6-di-tert-butylphenol)[1], common name Ethyl 702, is described. Improved analytical results are obtained using a mass flow controller (MFC) to regulate the CI reagent gas flow. The mass flow control approach is described as is the Continuum mode data collection ability of the PerkinElmer TurboMass™ GC/MS software.

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Experimental

The analysis of Ethyl 702 was performed by positive chemical ionization GC/MS using ammonia as the reagent gas. The sample was diluted in methylene chloride prior to analysis. A sample volume of 0.5 µL was injected into a capillary injector using the pressure pulse technique. Table 1 shows the full GC/MS method. The CI reagent gas flow was controlled using an Ethernet based mass flow controller at a rate of 0.8 mL/min which resulted in a vacuum of 3.7×10^{-4} Torr. The electron energy was set to 30 eV.

Table 1. Clarus SQ 8C GC/MS conditions.

Gas Chromatograph	PerkinElmer Clarus® 680		
Analytical Column	DB-5 (20 m x 0.18 mm id x 0.40 µm)		
Injection Port Type	Capillary Split/Splitless Injector		
Injector Temperature	300 °C		
Injection Type	Pressure Pulse		
Syringe Volume	5 µL		
Injection Volume	0.5 µL		
Injection Speed	Normal		
Carrier Gas	Helium @ 1.0 mL/min constant flow mode		
Split Flow	50 mL/min @ 0.5 min		
Oven Program	Temperature Hold	Time	Rate
	40 °C	0.5 min	25 °C/min
	200 °C	0 min	10 °C/min
	280 °C	0.1 min	End

Mass Spectrometer	PerkinElmer Clarus SQ 8C with MFC
GC Transfer Line Temperature	280 °C
Ion Source Temperature	150 °C
Ionization Mode	CI+
Function Type	Continuum
Electron Energy	-30 eV
Solvent Delay	0 – 2.00 min
Scan Range	*m/z* 60 – 700
Scan Time	0.25 sec
Interscan Delay	0.05 sec
Detector Voltage	1700 V

Discussion

Chemical Ionization

In Chemical Ionization (CI) analyte ions are formed through bimolecular reactions with ionized reagent gas molecules. The overall process is usually described as a three step process[2]:

1. Primary ion formation through reaction of reagent gas with incident electrons. This step is similar to standard Electron Impact ionization. Common reagent gasses include methane, isobutane, and ammonia.

$$X + e^- \rightarrow X^{+\bullet} + 2e^- \quad \text{(Reaction 1)}$$

2. Secondary reagent ion formation through reaction of ionized reagent gas molecules with other reagent gas molecules. The reagent gas is at a significantly increased partial pressure in the ion volume and will therefore undergo several transformations as it continues to interact with itself. The resulting plasma for methane, for example, extends the reaction experienced in EI, Reaction 2, to include the formation if many other ions and radicals, reactions 3 through 7[2]. This process is similar for other reagent gasses.

$$CH_4 + e^- \rightarrow CH_4^{+\bullet}, CH_3^+, CH_2^{+\bullet}, CH^+, C^{+\bullet}, H_2^{+\bullet}, H^+ \quad \text{(Reaction 2)}$$

$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet \quad \text{(Reaction 3)}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_7^+ \rightarrow C_2H_5^+ + H_2 \quad \text{(Reaction 4)}$$

$$CH_2^{+\bullet} + CH_4 \rightarrow C_2H_4^{+\bullet} + H_2 \quad \text{(Reaction 5)}$$

$$CH_2^{+\bullet} + CH_4 \rightarrow C_2H_3^+ + H_2 + H^\bullet \quad \text{(Reaction 6)}$$

$$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2 \quad \text{(Reaction 7)}$$

$$C_2H_5^+ + CH_4 \rightarrow C_3H_7^+ + H_2 \quad \text{(Reaction 8)}$$

3. Production formation through one of several reaction pathways with ionized species. In general there are four classes of reactions which result in product ion formation including:

Proton transfer:

$$M + [BH]^+ \rightarrow [M+H]^+ + B \quad \text{(Reaction 9)}$$

Electrophilic addition:

$$M + X^+ \rightarrow [M+X]^+ \quad \text{(Reaction 10)}$$

Anion extraction:

$$M + X^+ \rightarrow [M\text{-}A]^+ + AX \quad \text{(Reaction 11)}$$

Charge exchange:

$$M + X^{+\bullet} \rightarrow M^{+\bullet} + X \quad \text{(Reaction 12)}$$

Overall, CI is a "soft" ionization technique. The bimolecular reactions described in step 3 above result in substantially less fragmentation than would be expected from EI because the energy transfer is typically only a few eV or less, compared to the typical 70 eV used in EI. The molecular ion is a prominent feature of most CI spectra, even if the EI spectrum does not contain it. The prevalence of the molecular ion, in fact, makes CI a powerful complementary technique to use when added compound identification is required and why it is the ionization technique of choice for satisfying REACH requirements.

Optimizing Your GC/MS for Chemical Ionization

The following guidelines may be used to optimize your CI-enabled Clarus GC/MS system.

Reagent gas considerations – When following standard good laboratory practice, it is common to use high quality gas, regulators, and tubing fitted with the appropriate filters. This is especially true when performing GC/MS due to the added sensitivity of the mass spectrometer. It is therefore recommended to fit your CI reagent gas line with a suitable reagent gas filter as well. This is especially important because of the increased partial pressures of the reagent gas. When using a poor quality gas without a filter, it is possible during the product ion formation step above, to form ions from the impurities in the reagent gas. The main exception to this rule is ammonia as there is no recommended filter for ammonia gas.

Another consideration is the partial pressure of the CI reagent gas in the ion volume. Increasing the reagent gas flow increases the partial pressure in the ion volume. As described above, the partial pressure regulates how ionization proceeds and which and in what distribution possible reagent and secondary ions are present. Depending on the resulting spectra desired, the flow of the reagent gas into the ion volume may be adjusted accordingly. Using the new MFC hardware, flow rate settings near 1 mL/min are common.

Electron energy – Another parameter available to regulate the CI process is the electron energy. The accepted standard for EI mode operation is 70 eV. With CI, on the other hand, it is common to achieve satisfactory analytical results at much lower settings. The specifics of this tuning will be instrument and compound dependent; for the Clarus SQ 8 line, an electron energy of 35 eV or lower is common and can be optimized for the target analytes.

Mass Flow Controller Option

The MFC hardware is a drop-in replacement for the manual needle valve which is standard on all Clarus SQ 8C and Clarus 600 mass spectrometers. It is configured and controlled using a simple Ethernet interface. This control allows pushbutton operation along with real-time data plots. Its great advantage is the simplicity it brings to controlling the CI mechanism. As described above, chemical ionization can be complex to set up properly and is often viewed as an expert technique. Using this new hardware standardized procedures which define the specific CI reagent gas flow may be generated by senior laboratory personal that can then be reliably reproduced by less experienced users while maintaining data integrity. This is also a great advantage for sharing methods between laboratories to ensure consistency of results.

The control scheme is shown in Figure 1. Once configured, the desired flow rate is simply entered into the "Flow Setpoint" box and submitted. The electronic control will automatically adjust the CI reagent gas flow. In the Figure the red trace shows the set point flow rate while the blue trace shows the actual flow rate. The set flow rate is rapidly and reliably achieved.

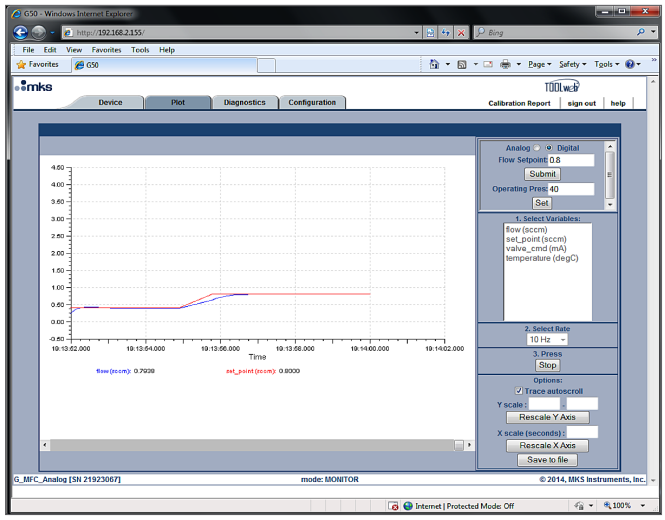

Figure 1. Java-based Ethernet control of the mass flow controller. In this example the flow was activated to 0.4 mL/min and 0.8 mL/min. The blue trace (flow readback) tracks the red trace (set point) closely.

The MFC control software contains calibration tables for the typical CI reagent gasses (methane, isobutane, and ammonia) and others. Switching between CI reagent gasses, therefore, is as simple as changing the source of gas at the rear of the instrument and selecting a different calibration table. It is important to note that the MFC hardware is completely compatible with corrosive compounds such as ammonia.

Ethyl 702 Results

Ethyl 702 was analyzed using ammonia positive CI under tuning conditions such that a prevalent EI spectrum was also present. The intent of this data is the positive identification of Ethyl 702 using both EI and CI characteristics. Full scan MS data were collected for library searchable spectra were acquired. The resulting spectrum is shown in Figure 2.

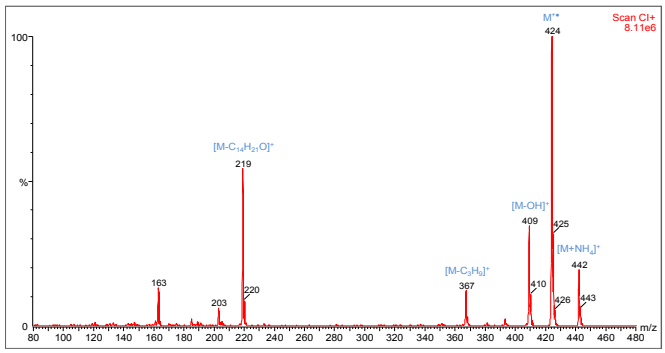

Figure 2. Continuum spectrum of Ethyl 702 peak.

The molecular ion peak, $M^{+\bullet}$, generated through the EI process, is clearly visible at *m/z* 424. So too is the ammonia adduct product, $[M+NH_4^+]$, at *m/z* 442 which is a results of the CI process. Although these are the result of separate ionization mechanism, their combination with the added confirmation of the correct chromatographic retention time, allows for the conclusive identification of Ethyl 702. This identification is confirmed performing a NIST library search; the results are shown in Figure 3. Although the data was collected in CI mode, matching to the EI spectra is quite good.

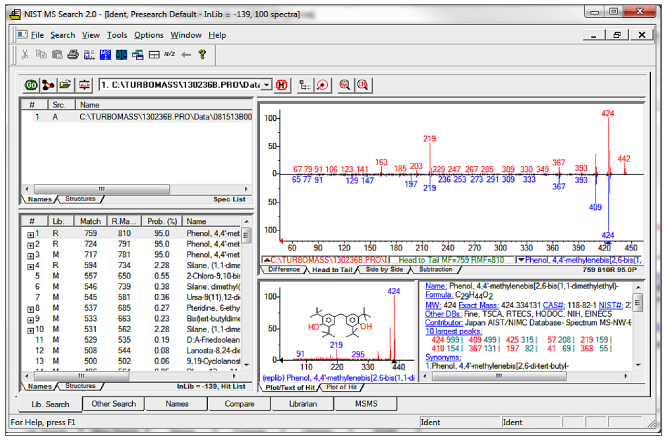

Figure 3. NIST library search results showing excellent matching.

A zoom of the ammonia adduct ion is shown in Figure 4. It is interesting to note that this data was collected using the continuum data format. This format, as opposed to the much more common centroid data format, is an analogue data format and provides rich insight into the performance of the ionization process and the instrument overall. The trade-off is the size of the data files which are many times larger than the equivalent centroid data file. Data collection in TurboMass GC/MS software is possible in centroid, continuum, or Multi Channel Analysis (MCA) as a standard feature. Selection is made in the MS Method editor as seen in Figure 5.

A more detailed analysis of Figure 4 demonstrates the utility of collecting data in continuum format. The first point to note is the symmetry of the individual mass peaks. Each peak is symmetric about the apex with no shouldering or other defects. The next point is the analysis of the resolution of the two ions. The valley between the peaks is sufficient to properly identify each. The final point is real baseline observed at all non-analyte peaks. The baseline is consistent and also free from defects. Taken together, these observations indicate the instrument is properly tuned and functional for this analysis. It would be difficult to fully discern the instrument operation from centroid data.

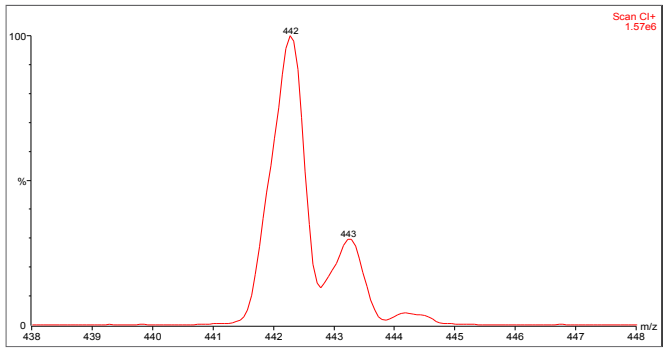

Figure 4. Zoom of ammonia adduct $[M+NH_4]+$ illustrating contoured nature of Continuum data.

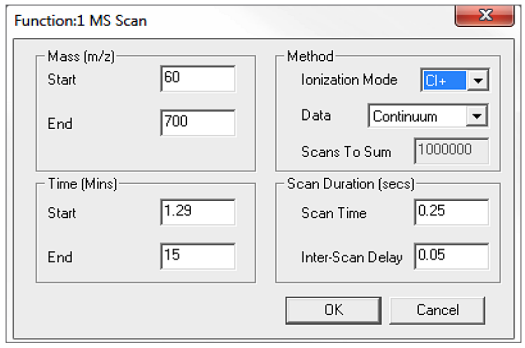

Figure 5. MS Scan Function settings available in TurboMass software. Choice of both ionization mode (EI, CI+, and CI-) and Data format (Centroid, Continuum, and MCA) are available.

It is for this reason that it is often recommended to collect data in continuum mode when instrument functionality is in question.

Continuum data may be utilized identically to centroid data. Both qualitative and quantitative analysis may be performed including library matching to centroid data. Additionally, within the TurboMass GC/MS software it is possible to calculate a centroid spectrum using the continuum data. This process is achieved by "manually centroiding" the data which includes smoothing, see Figure 6, and centering the data, see Figure 7. Figure 8 shows the adduct product ion spectrum from Figure 4 in approximated centroid format.

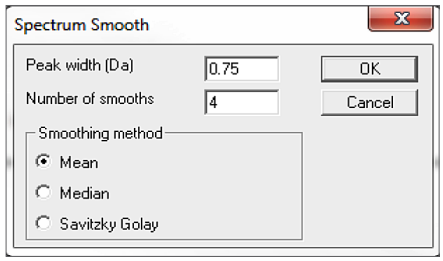

Figure 6. Spectrum smoothing options.

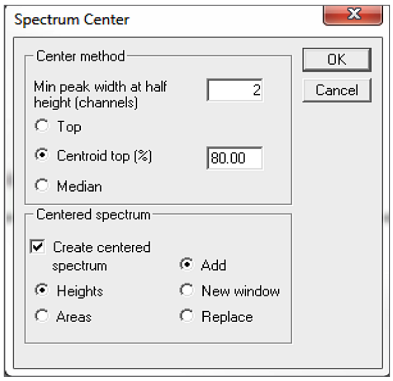

Figure 7. Spectrum Center options.

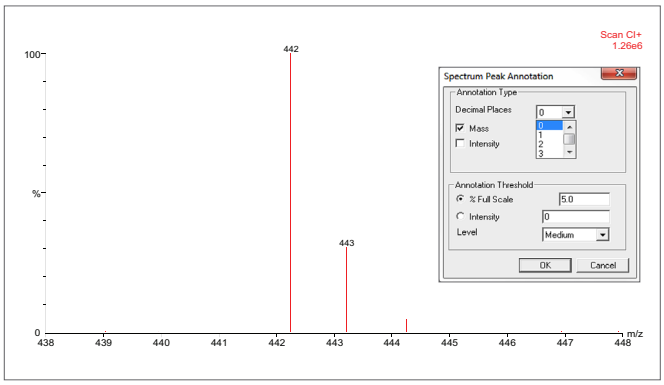

Figure 8. Calculated Centroid of the ammonia adduct peak shown in Figure 4 using parameters showing in Figures 6 and 7. In both Figure 4 and this Figure, the peak at *m/z* 444 is not labeled – this is intentional to demonstrate the ability within TurboMass to control the display. In this instance peak labeling is limited to peaks above a minimum threshold of 5% of the Base Peak intensity. See the inset for full options.

Conclusions

The positive identification of Ethyl 702 was described using chemical ionization gas chromatography mass spectrometry. The resulting spectra demonstrate positive identification of the respective compounds in fulfillment of REACH certification by using the ammonia adduct ion to confirm the compound molecular weight. Improved analytical results were obtained using a mass flow control (MFC) mechanism for the regulation of the CI Reagent gas flow. The mass flow control approach is described as is the Continuum mode data collection ability of the PerkinElmer TurboMass GC/MS software.

References

1. REACH registration can be found through the following link: http://apps.echa.europa.eu/registered/data/dossiers/DISS-dcee47d2-62ad-0171-e044-00144f67d031/DISS-dcee47d2-62ad-0171-e044-00144f67d031_DISS-dcee47d2-62ad-0171-e044-00144f67d031.html
2. A thorough treatment of CI can be found in Gross chapter 7. [: J. Gross, "Mass Spectrometry – A Textbook", 2nd edition, Springer, 2004, Chapter 7]

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