Application Note: 52282

Determination of DEHP in Culture Media by GC-MS/MS Using PCI Ammonia

Jessie Butler, Pat O'Brien, Stacy Crain, Thermo Fisher Scientific, Austin, Texas, USA
Viviane Yargeau, Ph.D., McGill University, Montreal, Canada

Key Words

- ITQ Ion Trap GC-MS^n
- TRACE GC Ultra
- Mass Frontier 7.0
- DEHP, Phthalates
- Positive Chemical Ionization

Introduction

Phthalates are industrial chemicals commonly added to plastics, usually to soften texture and improve flexibility. Because phthalates are widely used, exposure to these compounds is unavoidable. Unfortunately, phthalates are also suspected of being endocrine disrupting chemicals.[1] In animal studies, phthalates have caused abnormal effects in the male reproductive system.[2,3] Strong correlations appear in human studies as well.[4-6] Research is underway at McGill University and elsewhere to discover the mechanisms underlying the male reproductive toxicity of phthalates, and a simple, effective method of detecting phthalates in various samples would be advantageous to these researchers.

The goal of this application note is to introduce a method of elucidating the target phthalate, bis(2-ethlyhexl) phthalate (DEHP), in culture media by gas chromatography and tandem mass spectrometry (GC-MS/MS) using positive chemical ionization (PCI) with ammonia reagent gas. Phthalates are routinely analyzed in electron ionization (EI) with 149 *m/z* as the principal ion. Since all phthalates have similar spectra, it is difficult to identify which phthalate is present in a sample. By using PCI, the molecular ion is formed, which helps classify the phthalate.[7] Ammonia was selected as the chemical ionization reagent gas to increase the yield of the molecular ion. By choosing the proper reagent gas, PCI techniques can selectively protonate molecules and provide a high intensity of the quasimolecular ion for the MS/MS process.

Culture media samples with varying concentrations of DEHP were analyzed on a GC-MS system consisting of a Thermo Scientific TRACE GC Ultra gas chromatograph and a Thermo Scientific ITQ ion-trap series mass spectrometer. Structural elucidation was achieved by performing MS/MS on the molecular ion. Thermo Scientific Mass Frontier 7.0 software was then used to predict the theoretical fragments and fragmentation pathways of the detected product ion to confirm its identity.

Methods

Sample Preparation

Samples were centrifuged and the supernatant was recovered and extracted with an equal volume of chloroform. The pH of the supernatant was lowered below pH 2.0 with HCl prior to extraction. The organic phase of each sample was recovered for analysis by GC-MS. The culture media used to prepare the samples was Waymouth's MB 752/1 with 15% horse serum, 20 mM HEPES, and 0.25%

penicillin-streptomycin. DEHP was added to 10 mL of culture media to prepare each sample. A blank and five standards were used for the analysis of the two samples of different DEHP concentrations. Table 1 lists the contents of each sample.

Sample Number	Contents
GCMS #1	Culture media only
GCMS #2	Culture media containing DEHP (unknown concentration)
GCMS #3	Culture media containing DEHP (unknown concentration)
GCMS #4	Culture media with 1 µM DEHP
GCMS #5	Culture media with 5 µM DEHP
GCMS #6	Culture media with 10 µM DEHP

Table 1: Sample contents

Instrumental Analysis

A 1 µL splitless injection volume was programmed on the Thermo Scientific AS 3000 II autosampler, and a 45 second splitless injection was used. The TRACE GC Ultra™ was equipped with a standard split/splitless (SSL) injector, and the SSL injector temperature was set to 250 °C. A 5 mm i.d. splitless deactivated glass liner was used in the injector. The analytical column used was a Thermo Scientific TRACE TR-5MS 30 m × 0.25 mm i.d. × 0.25 µm film, which was installed 64 mm into the injection port. The ITQ™ mass spectrometer used for this analysis was configured with a 250 L/s turbomolecular pump. The complete method parameters can be found in Table 2.

Protonation is the most frequently used reaction in PCI. It leads to the formation of the quasimolecluar ion $(M + H)^+$, which can undergo fragmentation:

$$M + RH^+ \rightarrow MH^+ + R$$

Of the reagent gases available, ammonia and isobutene are typical soft CI gases, producing a higher yield of the quasimolecular ion $(M + H)^+$. In the method used for this analysis, ammonia was used at 2.0 mL/min at 10 psi source pressure. The ammonia gave full conversion to the $(M + H)^+$ molecular ion, which was ideal for MS/MS.

AS 3000 II	
Sample Volume (µL)	1
Viscous Sample	Yes
Sampling Depth in Vial	Bottom
Injection Depth	Standard
Pre-Inj Dwell Time (sec)	0
Post-Inj Dwell Time (sec)	0
Sample Rinses	1
Plunger Strokes	5
Pre-Injection Solvent Rinses	0
Post-Injection Solvent Rinses Solvent A (chloroform):	10

TRACE GC Ultra	
Oven Method	
Initial Temp (°C)	40
Initial Time (min)	2.0
Rate (°C /min)	20
Final Temperature (°C)	300
Final Hold Time (min)	5.0
SSL Method	
Temperature (°C)	250
Mode	Splitless
Split Flow	50
Constant Septum	on
Inject	1.0
Carrier Flow (mL/min)	1.0
Gas Saver	off
Vacuum Compensation	on
Transfer Line (°C)	300

ITQ	
Source Temp (°C)	250
Buffer Gas (mL)	0.3
Ammonia (mL)	2.0
DEHP Start Time (min)	6.0
Full Scan (*m/z*)	100–250
MS/MS Precursor (*m/z*)	391
Isolation Width (amu)	4.0
Isolation Time (ms)	12
Collision Energy (v)	0.75
Maximum Excitation Energy (v)	0.225
Excitation Energy Time (ms)	15
Product Ion Mass Range (*m/z*)	150–410
Product Ions (*m/z*)	181,184,278

Table 2: Instrument method for the full scan and MS/MS analysis of DEHP

Sample Processing and Result Derivation

First, one of the standards spiked in culture media (GCMS #2) was run in EI full scan. The spectrum is shown in Figure 1. Note the 149 *m/z* base ion.

When methane was used as the reagent gas, the PCI spectrum shows the formation of the molecular ion (391 *m/z*) but did not produce significant differences when compared to the full scan EI spectrum (Figure 2).

When ammonia was used in PCI, the spectrum contained only the molecular ion (391 *m/z*), as shown in Figure 3.

To generate the product ion spectrum, the MS/MS method outlined in Table 2 was used on the sample. The product ion spectrum is shown in Figure 4.

Mass Frontier™ 7.0 software was used to annotate the various fragment ions formed in the MS/MS analysis for DEHP ($C_{24}H_{38}O_4$) and confirm the identity of the detected product ions. Two of the fragment ions found are shown in Figures 5 and 6 along with the fragmentation pathways proposed by Mass Frontier 7.0 software.

The calibration curve for DEHP in MS/MS, obtained using GCMS #4–6, is shown in Thermo Scientific Xcalibur Quan Browser in Figure 7.

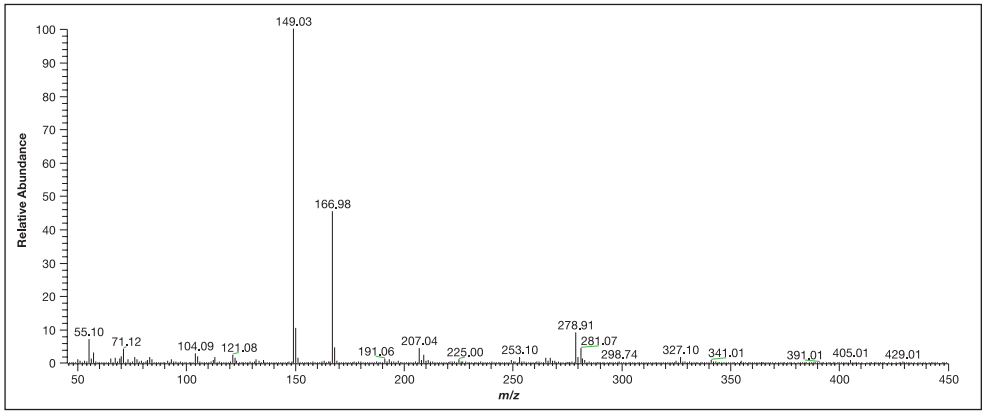


Figure 1: EI spectrum of DEHP in culture media in GCMS #5

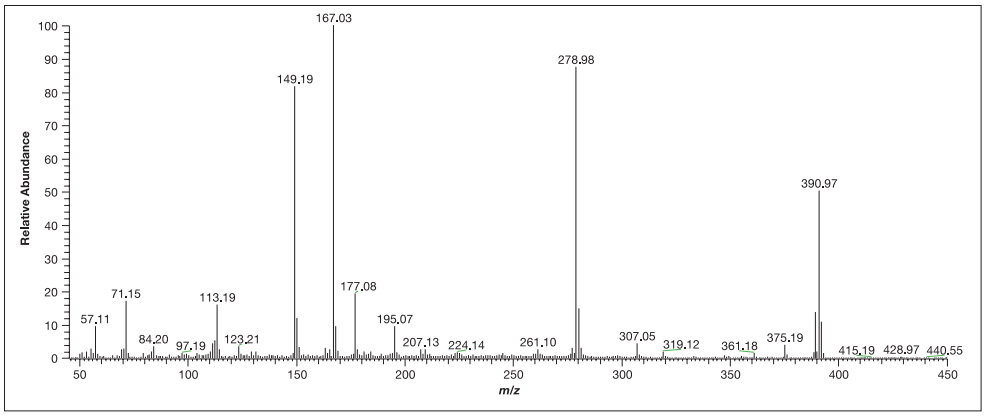


Figure 2: DEHP spectrum in PCI with methane at 0.8 mL/min

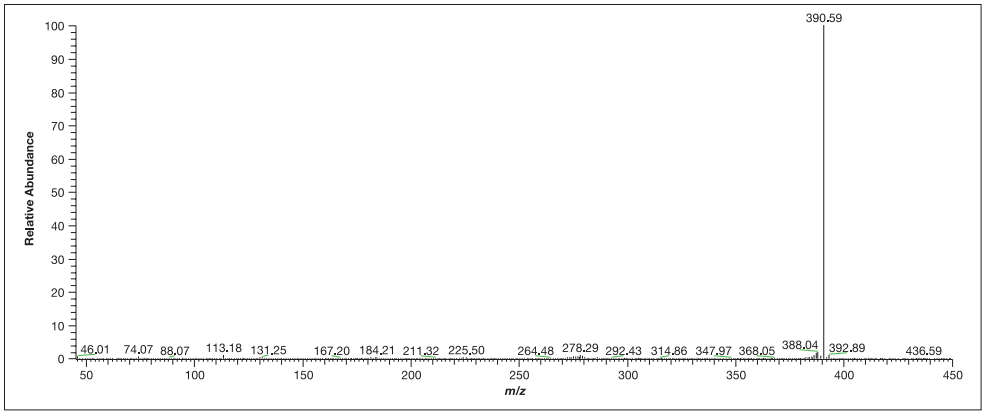


Figure 3: Spectrum of DEHP in PCI with ammonia at 2 mL/min at 10 psi

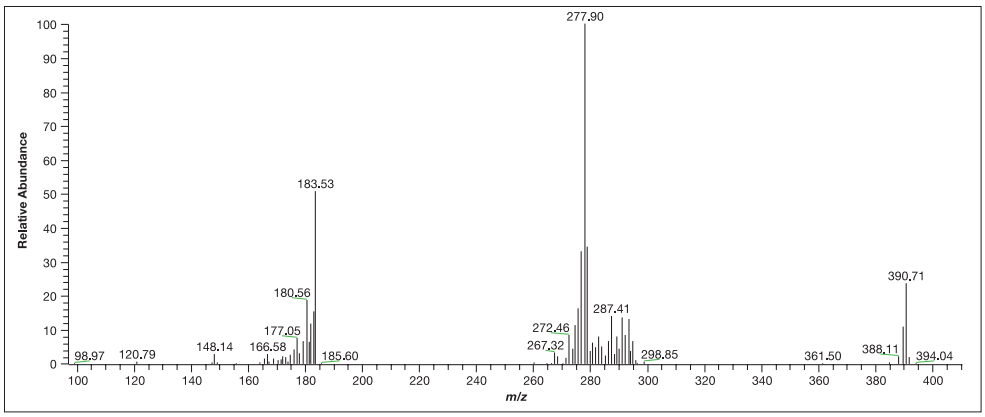


Figure 4: Product Ion Spectrum of DEHP

Figure 5: Mass Frontier proposed fragmentation pathway for product ion 180.56 *m/z*

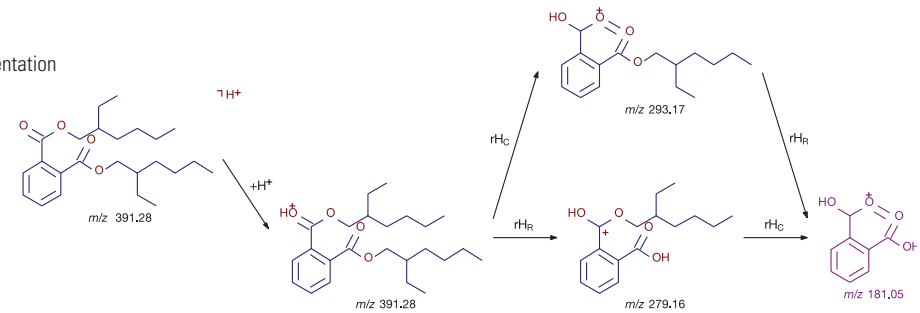
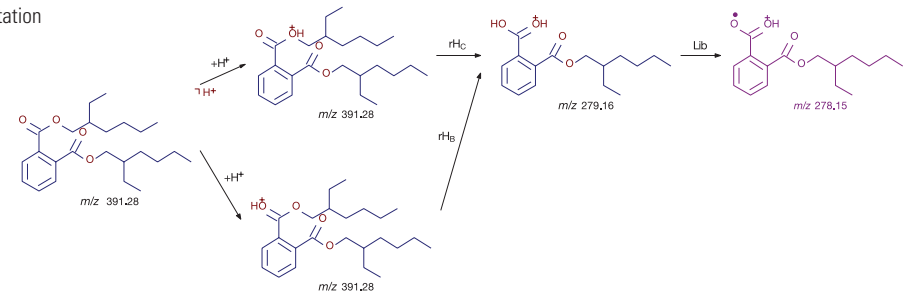


Figure 6: Mass Frontier proposed fragmentation pathway for product ion 277.90 *m/z*



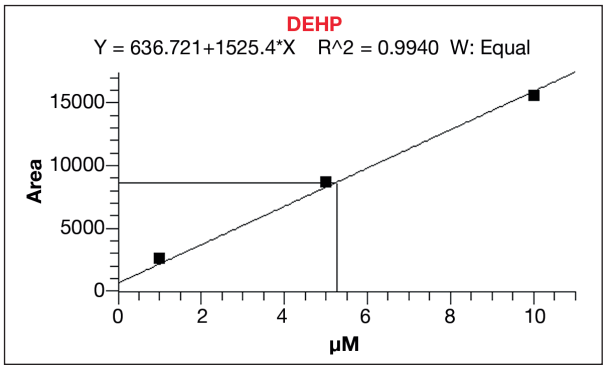


Figure 7: Calibration curve for DEHP in serum showing linearity using MS/MS

Results

The calibration curve (1, 5, 10 µM) run in culture media (samples GCMS #4–6) and processed in Xcalibur™ software by external standardization by MS/MS shows excellent linearity.

Two unknown samples were analyzed, GCMS #2 and GCMS #3. Both samples consisted of culture media containing DEHP. The quantitation results were GCMS #2: 5.3 µM (Figure 8) and GCMS #3: 0.3 µM.

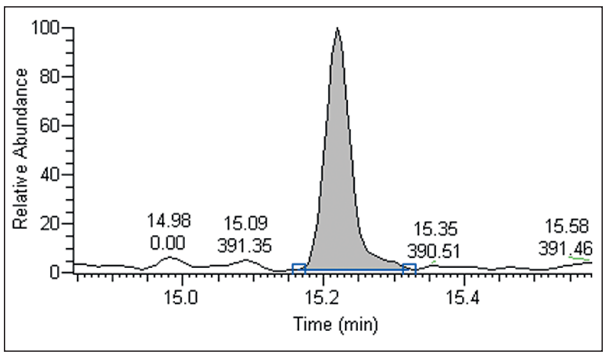


Figure 8: Integrated DEHP peak from sample GCMS #2 at 5.3 µM with a signal to noise ratio of 490:1

Conclusion

Phthalates are routinely analyzed in EI with 149 *m/z* as the principal ion. Since all phthalates have similar spectra, it is difficult to identify which phthalate is present in a complex sample. PCI MS/MS with ammonia was shown to have several advantages when used to identify phthalates. PCI is a highly selective ionization and produces a simpler spectrum of the molecular ion with high intensity. Coupled with MS/MS, the matrix interferences from biological samples were eliminated, enabling quantitation in the low µM levels in culture media. The ability of this method to isolate DEHP from other phthalates makes it very useful to those researching pharmacokinetic effects of this particular phthalate in animal and human studies.

Of the reagent gases available, ammonia, a typical soft CI gas, produced a higher yield of the quasimolecular ion $(M + H)^+$. Structural elucidation was achieved by performing MS/MS analysis on the molecular ion. The TRACE GC Ultra and ITQ ion trap mass spectrometer generated a linearity of $R^2 > 0.990$ from 1 to 10 µM. Two samples of unknown concentration were analyzed and concentrations of 5.3 and 0.3 µM were determined. Mass Frontier software was able to fully annotate the product ion spectra with fragment ion structures and provide fragmentation pathways for the given molecular structures. Mass Frontier software also proved to be a valuable tool in the confirmation of product ions formed in PCI MS/MS for DEHP.

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