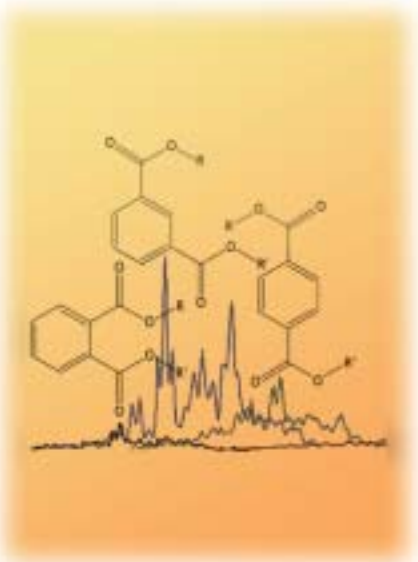
# Determination of Phthalate Esters by Positive Chemical Ionization MS with Retention-Time Locked GC



**The authors describe a new instrumental method for the determination of phthalate esters using positive chemical ionization and retention-time locking gas chromatography. Positive chemical ionization with ammonia provides a high degree of selective ionization for the phthalates, primarily producing spectra in which the protonated molecule is the base peak. This method provides easy discrimination among the phthalates based upon their molecular weights, and retention-time locking enhances confidence in the identification of the various isomers. In their approach, the authors considered both pure compounds and technical mixtures.**

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The widespread use and manufacture of plastics have made the phthalate esters one of the most ubiquitous compound classes in the environment. As plasticizers, phthalates enhance polymer flexibility through their function as intermolecular lubricants. Because they are additives and not reagents, they are not bound chemically in a polymer and are available to leach from matrices.

Phthalates are components of cosmetics, detergents, lubricating oils, polychlorinated biphenyl substitutes, carriers in pesticide formulations, solvents, and building products such as flooring, sheeting, and films. Consequently, the potential for human exposure is high. Toxicological studies have linked some of these compounds to liver and kidney damage and to possible testicular or reproductive-tract birth defect problems, which classify them as endocrine disrupters. Scientists at the U.S. Centers for Disease Control (Bethesda, Maryland) for the first time have documented human exposure to phthalates by determining monoester metabolites in human urine (1). Their work leads to the conclusion that "phthalate exposure is both higher and more common than previously suspected" (1). Of particular concern to Blount and co-workers (1) were the significantly higher concentrations of the dibutyl phthalate metabolite in the urine of childbearing-age women (20–40 years) than in that of other segments of the population.

The presence of phthalate esters in polyvinyl chloride (PVC) toys has generated the most controversy. Regulators in Greece have completely banned soft PVC toys, and Austria, Denmark, Finland, France, Germany, Norway, and Sweden have banned phthalates in PVC toys for children less than three years old. In December 1999, the European Union (EU), concerned with a "serious and immediate risk" to children, placed an emergency ban on six of the phthalate esters in soft PVC toys and childcare products meant to be placed in the mouths of children less than three years old (2). None of the six banned phthalates can exceed 0.1% by weight. A recent study suggests a connection between high concentrations of phthalate esters in serum and the incidence of premature breast development in females less than eight years old in Puerto Rico (3).

These heightened concerns suggest the need for an improved method of detecting and characterizing phthalate esters that can be applied to various matrices.

## Phthalate Structure and Mass Spectra

Figure 1 shows the generalized structures of the three phthalates. By far, the most commonly used phthalate esters are based on the 1,2-benzenedicarboxylic acid structure (Figure 1a). An almost infinite number of potential side chains (R) and combinations of the side groups (R and R′) are possible. The phthalates can be symmetrical (R = R′) or asymmetrical (R ≠ R′). For 1,2-phthalate esters with saturated alkyl side chains (without oxygen), the most intense peak in the electron ionization mass spectrum at 70 eV always appears at *m/z* 149 because of the rapid formation and stability of the ion shown in Figure 2. (The only exception is R = R′ = $CH_3$, where the base peak is at *m/z* 163.)

Invariably, the molecular ion is very weak or altogether absent, and other fragments that provide information about the phthalate identity also are of low abundance. As an example, consider the electron ionization mass spectrum of di-*n*-butyl phthalate, one of the six phthalates banned by the EU, and bis(4-methyl-2-pentyl) phthalate depicted in Figure 3. The identifying fragments have relative intensities of less than 10%. Gas chromatography (GC) provides some separation of the phthalates, but distinguishing the individual phthalates of concern is difficult with the array of possible isomers and only one identifying ion (*m/z* 149). The situation is complicated in sample matrices in which the background can obscure important fragment ions.

Analysts can make more-confident identifications of phthalate esters using positive chemical ionization mass spectrometry (MS) with retention-time locking GC. Chemical ionization provides a more-selective form of ionization than electron impact (4). By choosing the reagent gases judiciously, analysts have greater control of the degree of compound fragmentation.

Retention-time locking is a feature that enables the replication of analyte retention times produced by an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmington, Delaware) within seconds by any other identical gas chromatograph using the same GC method (5–7). This locking is achieved by correlating the retention time ($t_R$) of a reference analyte, called the *locking compound*, with the carrier-gas pressure. After column replacement or service, workers can use this correlation to set the carrier-gas pressure so that it precisely reproduces the locking compound retention time and,

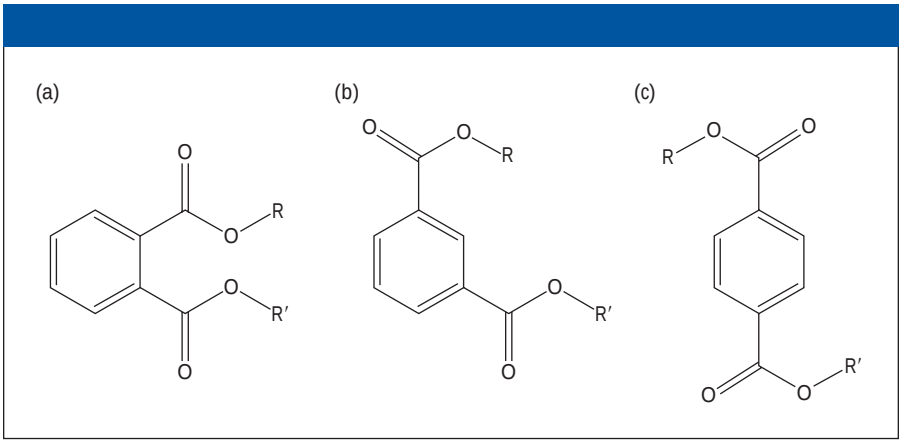


**Figure 1:** Structures of (a) phthalic ester (the 1,2-benzenedicarboxylic acid ester), (b) isophthalic ester (the 1,3-benzenedicarboxylic acid ester), and (c) terephthalic ester (the 1,4-benzenedicarboxylic acid ester). R and R′ represent alkyl side chains that may be symmetrical or asymmetrical, linear or branched, saturated or unsaturated, and may or may not contain oxygen.

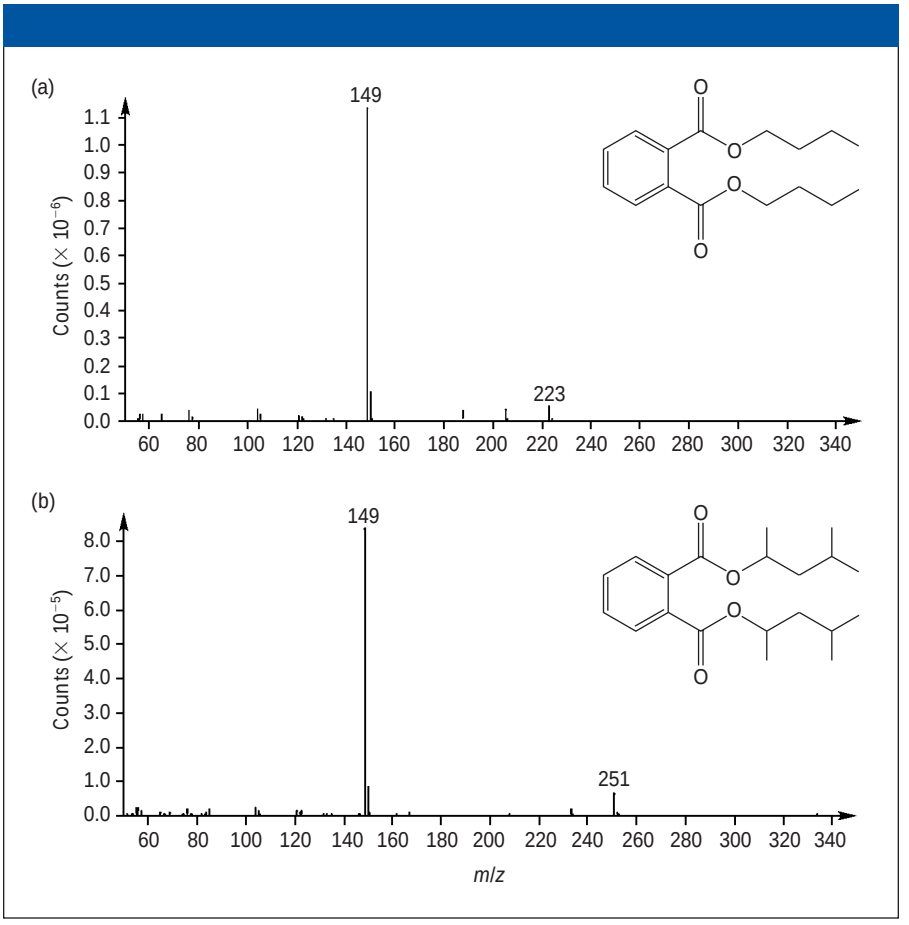


**Figure 3:** Electron impact ionization mass spectra of (a) di-*n*-butyl phthalate and (b) bis(4-methyl-2-pentyl) phthalate from *m/z* 50 to *m/z* 350 at 70 eV. The nominal molecular masses are 278 and 334, respectively.

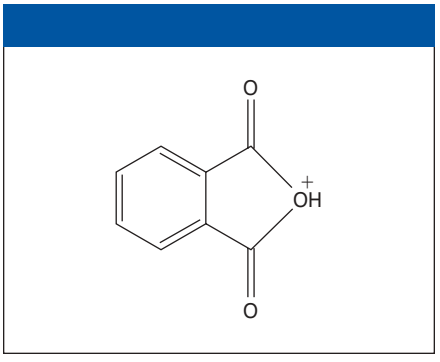


**Figure 2:** Structure of the ion at *m/z* 149, the most abundant ion in the electron ionization mass spectra of the phthalate esters with saturated alkyl side chains.

therefore, the retention times of all analytes in a method.

Retention-time locking is especially useful for GC–MS acquisitions in selected-ion monitoring mode, which can be difficult to maintain with many ion groups because retention-time locking removes changes in compound retention times (6). Similarly, methods that quantitate a large number of analytes benefit from retention-time locking because compound retention times become permanent and do not require constant updating to reflect changes caused by column servicing. Retention-time locking relies upon a gas chromatograph's precise and stable electronic pressure and oven control and upon highly uniform, stable, and reproducible capillary columns. The entire retention-time locking procedure was automated and included in the GC–MS ChemStation software (G1701 BA and higher, Agilent Technologies).

Retention-time locking simplifies compound identification because it enables the use of standardized GC methods that can be reproduced in any laboratory. It also allows the compiling and referencing of universal compound retention-time databases. For example, the retention times of the 29 phthalates studied in our work can be replicated on any Agilent 6890 gas chromatograph and used to identify phthalates, even though users do not have a complete, in-house set of phthalates.

## Experimental

**Chemicals:** We obtained phthalate esters from Ultra Scientific (North Kingstown, Rhode Island); AccuStandard (New Haven, Connecticut); and ChemServices (West Chester, Pennsylvania) as neat compounds and mixtures. Dilutions were made in isooctane from Burdick & Jackson (VWR Scientific, West Chester, Pennsylvania).

The positive chemical ionization reagent gas purities were 99.99% or higher.

**Instrumentation:** We used Agilent 6890 Plus gas chromatographs in standard 120-V or faster-ramping 220-V versions, an Agilent 7693 Automatic Liquid Sampler autosampler, and an Agilent 5973N mass-selective detector with a chemical ionization option. We performed pulsed, splitless injections using an injection port temperature of 300 °C with a rapid pressure program to 25 psi with a 1-min hold. The oven temperature program was 80 °C for 1.00 min, increased to 200 °C at 50.00 °C/min, then increased to 350 °C at 15.00 °C/min, and held for 2.00 min. The oven equilibrium time was 0.25 min. The mass-selective detector transfer line temperature was 325 °C.

We used a DB-5MS 30 m × 0.25 mm, 0.25-μm $d_f$ GC column (Agilent Technologies, Folsom, California). The nominal helium carrier-gas flow was 1.2 mL/min.

Our system also included septa that could withstand 400 °C temperatures; deactivated, single-taper 4-mm i.d. liners; a Vespel 250-μm GC column ferrule; and a 0.4-mm i.d. graphitized Vespel mass-selective detector interface ferrule.

In the mass spectrometer, we used positive chemical ionization automatic tuning parameters for methane. The electron multiplier voltage was the automatic tuning parameter plus 400 V. The solvent delay was 4.00 min, and the scan parameters were set appropriate to the chemical ionization gas (for example, *m/z* 194–550 for ammonia). The quadrupole and source temperatures were 150 and 250 °C, respectively. The ammonia gas flow rate was 0.5 mL/min (10% of maximum).

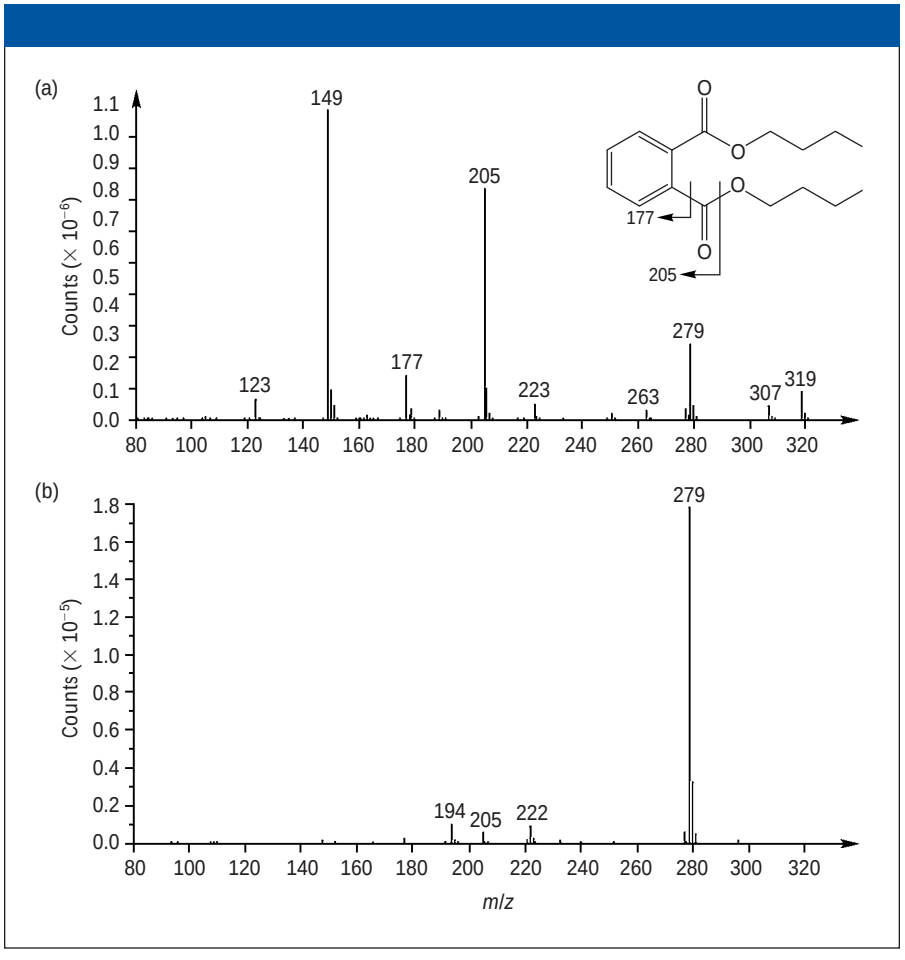


**Figure 4:** Positive chemical ionization (a) methane and (b) ammonia mass spectra of di-*n*-butyl phthalate.

## Results and Discussion

The relatively vigorous fragmentation produced by methane reagent gas generates positive chemical ionization spectra for the dialkyl phthalate esters that still resemble their electron ionization spectra but with additional ions that correspond to the protonated molecule $[M + H]^+$ and adducts $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$. In most cases, the fragment at *m/z* 149 is the base peak, but the useful identifying ions at *m/z* M + 1, M + 29, and M + 41 are relatively intense with $[M + H]^+$ from 10% to 30% (Figure 4). Another intense identifying ion in the spectra of the dialkyl 1,2-phthalate spectra is caused by a fragment that corresponds to the loss of one of the alkyloxy side chains (loss of −OR) to produce an ion that is analogous to that of Figure 2 with the R-side chain replacing H on oxygen. As the length of the ester alkyl chain increases, the intensity of this fragment decreases. This fragment is most intense for the dimethyl and diethyl 1,2-phthalates and for the dibutyl and dipentyl (diamyl) phthalates, approximately 75% of the *m/z* 149 base peak. (Apparently, the loss of the alkyl side chain *not* accompanied by the oxygen might

**Table I: Retention-time locked and MS data for phthalate ester analytes***

| Name | CAS Number | Molecular Mass | Formula | Positive Chemical Ionization MS Base Peak† (*m/z*) | Retention Time* (min) |
|---|---|---|---|---|---|
| Dimethyl phthalate | 131-11-3 | 194 | $C_8H_4O_4(CH_3)_2$ | 195 | 4.32 |
| Dimethyl isophthalate‡ | 1459-93-4 | 194 | $C_8H_4O_4(CH_3)_2$ | 212 | 4.54 |
| Diethyl phthalate | 84-66-2 | 222 | $C_8H_4O_4(C_2H_5)_2$ | 223 | 4.81 |
| Diethyl terephthalate‡ | 636-09-9 | 222 | $C_8H_4O_4(C_2H_5)_2$ | 240 | 5.06 |
| Benzyl benzoate§ | 120-51-4 | 212 | $C_{14}H_{12}O_2$ | 230 | 5.62 |
| Diisobutyl phthalate | 84-69-5 | 278 | $C_8H_4O_4(C_4H_9)_2$ | 279 | 5.95 |
| Di-*n*-butyl phthalate‖ | 84-74-2 | 278 | $C_8H_4O_4(C_4H_9)_2$ | 279 | 6.40 |
| Bis(2-methoxyethyl) phthalate | 117-82-8 | 282 | $C_8H_4O_4(C_2H_4OCH_3)_2$ | 283 | 6.57 |
| Diamyl phthalate | 131-18-0 | 306 | $C_8H_4O_4(C_5H_{11})_2$ | 307 | 6.94 |
| Bis(2-ethoxyethyl) phthalate | 605-54-9 | 310 | $C_8H_4O_4(C_2H_4OC_2H_5)_2$ | 311 | 7.13 |
| Butyl benzyl phthalate‖ | 85-68-7 | 312 | $C_8H_4O_4(C_4H_9)(CH_2C_6H_5)$ | 313 | 8.42 |
| Diphenyl phthalate | 84-62-8 | 318 | $C_8H_4O_4(C_6H_5)_2$ | 319 | 9.45 |
| Diphenyl isophthalate | 744-45-6 | 318 | $C_8H_4O_4(C_6H_5)_2$ | 319 | 10.30 |
| Dicyclohexyl phthalate | 84-61-7 | 330 | $C_8H_4O_4(C_6H_{11})_2$ | 331 | 9.32 |
| Bis(4-methyl-2-pentyl) phthalate | 146-50-9 | 334 | $C_8H_4O_4(CH_3C_5H_{10})_2$ | 335 | 6.93 |
| Diisohexyl phthalates# | 146-50-9 | 334 | $C_8H_4O_4(C_6H_{13})_2$ | 335 | 7.55–8.28 |
| Dihexyl phthalate | 84-75-3 | 334 | $C_8H_4O_4(C_6H_{13})_2$ | 335 | 8.34 |
| Dibenzyl phthalate | 523-31-9 | 346 | $C_8H_4O_4(CH_2C_6H_5)_2$ | 347 | 10.51 |
| Hexyl-2-ethylhexyl phthalate | 75673-16-4 | 362 | $C_8H_4O_4(C_2H_5C_6H_{12})(C_6H_{13})$ | 363 | 8.84 |
| Bis(2-*n*-butoxyethyl) phthalate | 117-83-9 | 366 | $C_8H_4O_4(C_2H_4OC_4H_9)_2$ | 367 | 8.98 |
| Bis(2-ethylhexyl) phthalate‖ | 117-81-7 | 390 | $C_8H_4O_4(C_2H_5C_6H_{12})_2$ | 391 | 9.32 |
| Di-*n*-octyl phthalate‖ | 117-84-0 | 390 | $C_8H_4O_4(C_8H_{17})_2$ | 391 | 10.28 |
| Dioctyl isophthalate‡ | 137-89-3 | 390 | $C_8H_4O_4(C_8H_{17})_2$ | 408 | 10.84 |
| Diisononyl phthalates# | 28553-12-0 | 418 | $C_8H_4O_4(CH_3C_8H_{17})_2$ | 419 | 9.4–11.10 |
| Dinonyl phthalate | 84-76-4 | 418 | $C_8H_4O_4(C_9H_{19})_2$ | 419 | 11.19 |
| Diisodecyl phthalates# | 26761-40-0 | 446 | $C_8H_4O_4(CH_3C_9H_{18})_2$ | 447 | 10.16–11.86 |
| Didecyl phthalate | 84-77-5 | 446 | $C_8H_4O_4(C_{10}H_{21})_2$ | 447 | 12.05 |
| Diundecyl phthalate | 3648-20-2 | 474 | $C_8H_4O_4(C_{11}H_{23})_2$ | 475 | 12.87 |
| Didodecyl phthalate | 2432-90-8 | 502 | $C_8H_4O_4(C_{12}H_{25})_2$ | 503 | 13.65 |
| Ditridecyl phthalates# | 119-06-2 | 530 | $C_8H_4O_4(C_{13}H_{27})_2$ | 531 | 12.01–13.81 |

*Retention times are locked relative to diphenyl phthalate at 9.450 min.
†Nominal base peak in the ammonia positive chemical ionization spectrum.
‡Unlike the 1,2-phthalates, the 1,3- and 1,4-alkyl esters often form the ammonia adduct $(M + 18)^+$.
§Benzyl benzoate is included because it is used as a surrogate in U.S. Environmental Protection Agency Method 8061.
‖ Indicates phthalates banned by the EU.
#Retention time ranges are given for the isoalkyl phthalate technical mixtures.

be a preferred route in the dialkyl isophthalates.)

Although positive chemical ionization with methane generates phthalate spectra that are richer in intense identifying ions of structural significance than electron ionization, methane positive chemical ionization can produce excessive chemical noise, which complicates analyte detection, in complicated matrices. By contrast, ammonia reagent gas may be more useful for complex samples. Ammonia, with its higher proton affinity, reduces chemical noise and provides very simple phthalate spectra (see Figure 4). This relatively gentle ionization produces protonation of the dialkyl 1,2-phthalates with *m/z* M + 1 as the base peak in their spectra. (Note that the ammonia positive chemical ionization spectra of alkyl isophthalates and terephthalates tend to display base peaks due to adduct formation in preference to protonation. A notable exception is the diphenyl isophthalate, probably for steric reasons.) Combining ammonia positive chemical ionization with retention-time locking simplifies phthalate identification.

Table I lists the phthalate ester names, Chemical Abstracts Service (CAS) numbers, molecular formulas, nominal molecular masses, base peaks in the ammonia positive chemical ionization spectrum, and elution times under the retention-time locking regime. These retention times are locked relative to diphenyl phthalate, which has been chosen as the retention-time locking standard and locked to be eluted at 9.450 min. The branched-chain isomers tend to be eluted before their straight-chain analogs on this column phase. Phthalate ester isomers, which produce identical positive chemical ionization spectra, can be distinguished by their locked retention times. Conversely, differing phthalate esters, which may be coeluted with other phthalates under these conditions, are differentiated by their characteristic ions.

Technical formulations of the isoalkyl phthalates contain a variety of alkyl side chains that differ in their branching (the nominal isoalkyl isomers) and in the number of carbons in their side chains. Because of lesser or greater numbers of $CH_2$ groups in the side chains, these differences result in additional ions at ± *m/z* 14, ± *m/z* 28, etc., around the mass of the nominal isoalkyl phthalate ion. For example, technical-grade diisononyl phthalate contains compounds that generate ions at *m/z* 391 (minor), *m/z* 405, *m/z* 433, and *m/z* 447 in addition to the nominal diisononyl phthalate compound at *m/z* 419, as Figure 5 shows. The gentle ionization of ammonia reagent gas, the elution times, and the study of the spectra of other pure isomers such as dinonyl phthalate suggest that these fragments are *not* formed by the positive chemical ionization process but reflect these different alkyl-side-chain impurities.

Figure 6 demonstrates the utility of the ammonia positive chemical ionization compared with conventional electron ionization analysis. The electron ionization spectra of the phthalates produced *m/z* 149 as the base peak for all the phthalates present; the other distinguishing ions were minor constituents (<10% relative intensity), which complicated identification. However, we could easily distinguish the various phthalates by examining the appropriate ammonia positive chemical ionization ions.

## Conclusions

An analytical method that combines positive chemical ionization using ammonia reagent gas with retention-time locked GC is an effective approach to determine phthalate esters. Using ammonia reagent gas in a mass spectrometer produces a simplified mass spectrum, which immediately allows the phthalate esters to be distinguished by their molecular weight. Locking the chromatography enables analysts to use retention time data to discriminate between phthalate ester isomers and to enhance their confidence in the identification.

The method is well suited to analyzing phthalates in complex matrices such as plastics, cosmetics, and environmental samples. The selective ionization generates a strong signal and minimizes ancillary fragmentation and chemical background. The ability to unambiguously discriminate and categorize the components of technical grade phthalates and phthalate mixtures also underscores this method's potential application in quality control testing and in characterizing commercial phthalate ester products by a unique retention-time locking GC–MS signature.

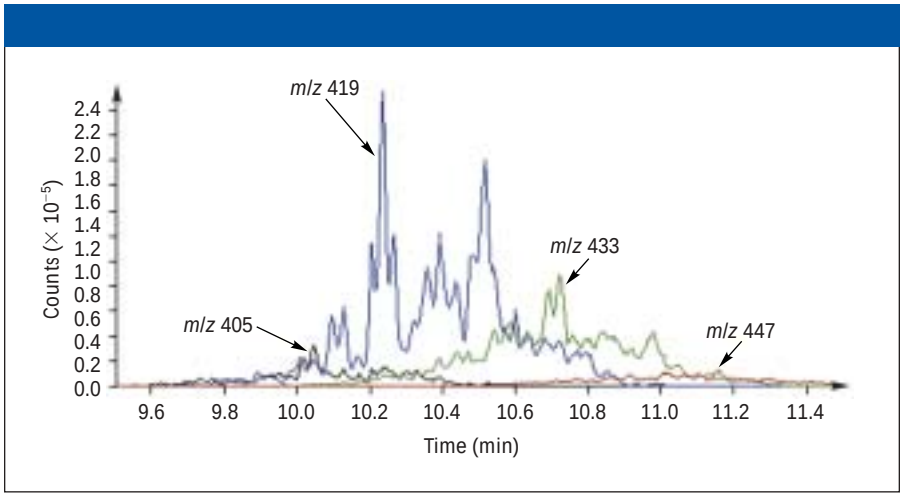


**Figure 5:** Ammonia positive chemical ionization extracted ion chromatogram of technical diisononyl phthalate. The diisononyl appears as the major component at *m/z* 419, and ions at *m/z* 405, *m/z* 433, and *m/z* 447 indicate alkyl chains shorter by one $CH_2$ unit and longer by one and two $CH_2$ units, respectively.

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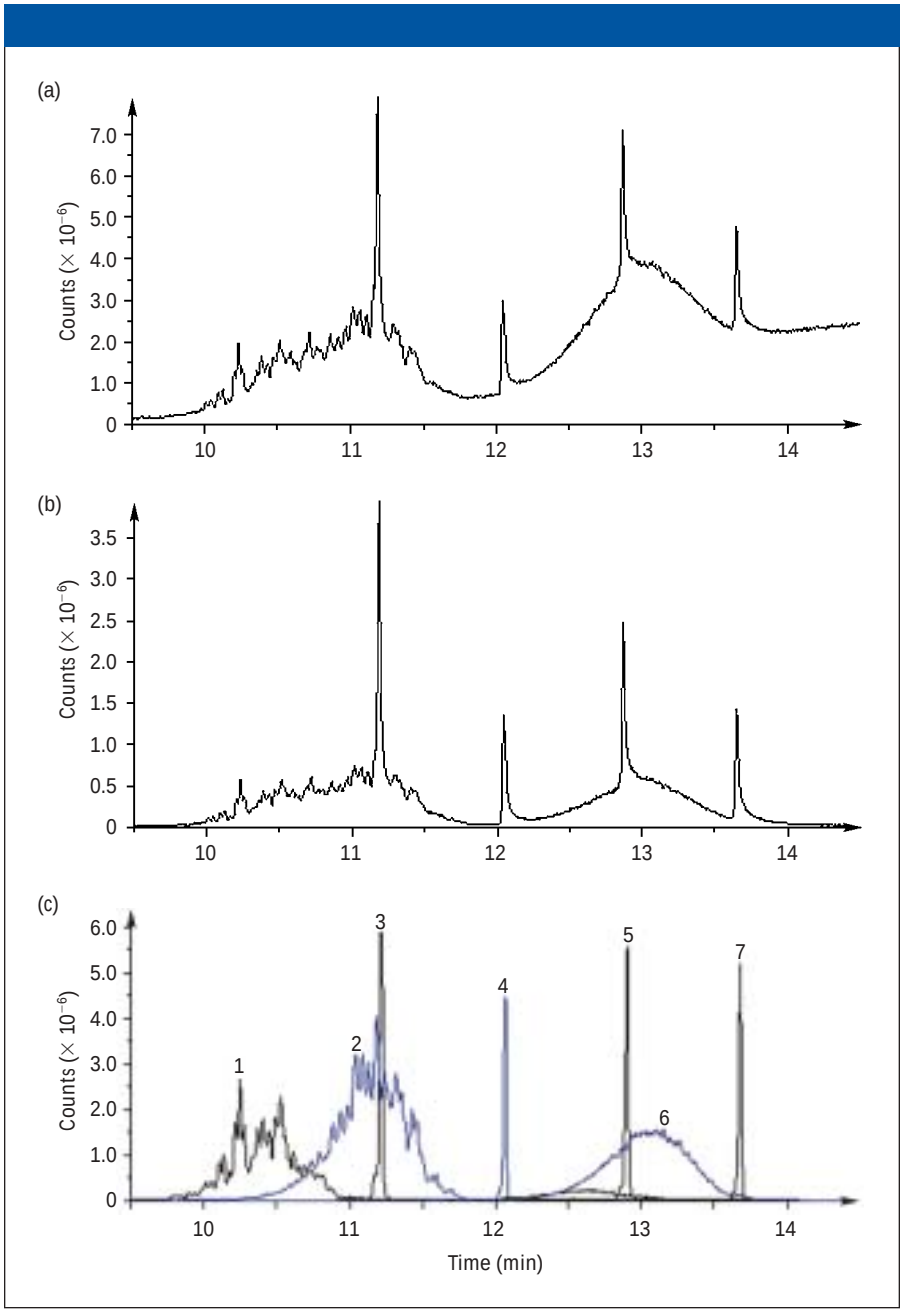


**Figure 6:** Analysis of phthalate esters using electron ionization and positive chemical ionization. Shown are (a) an electron ionization chromatogram, (b) an extracted ion chromatogram at *m/z* 149 obtained by electron ionization, and (c) a chromatogram obtained by positive chemical ionization with ions selected for the individual phthalate classes as listed in Table I. Phthalate ester peaks: 1 = diisononyl, 2 = diisodecyl, 3 = di-*n*-nonyl, 4 = di-*n*-decyl, 5 = di-*n*-undecyl, 6 = ditridecyl, 7 = di-*n*-dodecyl.