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Ionization Methods in Gas Phase Mass Spectrometry; Operating Modes of the 5973N*etwork* Series MSDs

Introduction

This note describes the salient features of the various mechanisms for producing ions in mass spectrometry. The purpose is to aid in the selection of the particular 5973N*etwork* MSD for the customer's interest and application.

Electron Ionization

The production of ions at low pressures by electric currents is at the very heart of mass spectrometry. The direct bombardment of analyte molecules by electrons is referred to as electron ionization (EI), which typically utilizes electrons at an energy of 70-eV. This energy has been universally chosen because it is well in excess of the energy required to ionize and fragment molecules. It is because all molecules can be fragmented by electron ionization that mass spectrometry is one of the few truly universal methods of compound detection and identification and that it has become a widely applied analytical technique. (Note that a feature of the 5973N MSD instruments is that they provides for user selection of the electron energy if the user wishes to explore fragmentation). Adoption of this 70-eV standard has allowed libraries to be created to identify molecules by their unique fragmentation patterns under electron impact. Fragmentation patterns produced by unknown compounds may be searched and identified through a series of algorithms and mass spectral libraries.

It is a salient feature of quadrupole mass spectrometer systems that they generate very stable and reproducible ion-fragmentation patterns. These are commonly called "classical" spectra because the fragmentation patterns that are produced reflect the ion's molecular composition. This is also useful when the user is interested in target compound analysis. Applying selected ion monitoring (SIM), the user selects a few ions representative of the compound of interest and monitors only those ions that indicate the presence of that compound. The high duty cycle for the SIM measurement and the very reproducible ion ratios provide confident quantitation of the analyte at very low concentrations.

The advantages of electron ionization on the 5973N MSDs include high sensitivity, classical fragmentation spectra, reproducible ion ratios, library searchable spectra, stable and reproducible SIM ion ratios, and applicability in all analytical markets.

Chemical Ionization

Early in the development of mass spectrometry, when pumping speeds and vacuum quality were low, "spurious" peaks were apparent in the resultant spectra due to reactions involving background gases. These "interferences" were deemed an annoyance and, as vacuum technology improved, they disappeared from spectra and consideration for roughly 50 years. In the 1960s researchers realized that these ion-molecule reactions in the ionization region provided unique information on analytes. Ionization of molecules by ion-molecule reactions is now commonly referred to as chemical ionization (CI). There are two important approaches to chemical ionization available on the 5973N MSDs: positive chemical ionization (PCI) and electron-capture negative ion chemical ionization (ECNI).

Positive Chemical Ionization

In direct contradiction of the struggle to achieve better vacuums for better EI mass spectrometry, chemical ionization mass spectrometry begins by intentionally adding a reagent gas to the ion source at pressures from about 0.1 Torr to 2 Torr. Electrons from the filament ionize the reagent gas to produce reagent ions (RH^+). In positive chemical ionization (PCI) it is these reagent ions that react with the analyte molecules (M) to produce ionization. Since the reagent pressures are relatively high, a variety of reagent ions may be produced and a variety of ionization mechanisms (ion-molecule reactions) are possible. Table 1 lists the most prevalent species produced in the source for the most common PCI reagent gases. Exactly what reactions are possible is determined by thermodynamics. The most commonly applied PCI reactions are proton transfer and adduct formation (or cluster ion formation):

proton transfer: $M + RH^+ \rightarrow (M+H)^+ + R$
adduct formation: $M + RH^+ \rightarrow (R+M+H)^+$

In proton transfer, whether the analyte molecule M acquires the proton from the reagent ion RH^+ or not depends on whether the analyte or the reagent molecule has the stronger affinity for the proton. This is similar to (Brønsted) acid-base chemistry in solution: the strongest base gets the proton or the strongest acid donates the proton, as determined by their relative affinities. This reveals the power of positive chemical ionization. By selecting reagent gases that produce ions with different proton affinities (or tendencies to donate a proton), the user can obtain some selectivity in what is ionized and in the degree of fragmentation. Whether a compound is protonated or not by proton transfer is determined by the relative affinities. The degree of fragmentation is determined by the *difference* in proton affinities; the larger the difference, the more fragmentation. Most organic compounds have proton affinities of the order of ~200 ± 30 kcal/mole. As a result, the greatest fragmentation is usually achieved with methane and the least with ammonia, the reason being that the differences in proton affinity are usually greatest for methane and least for ammonia (refer to Table 1). However, relative to electron impact, in which the energies involved are of the order of 7 to 10 times the ionization energies, chemical ionization is very "gentle" since the energy involved is comparable to bond dissociation energies. An example may make this more apparent.

Table 1. Reagent species and their properties for the common PCI gases.

Reagent Gas	Major Species	CI Products	Proton Affinity (kcal/mole)
Methane	CH_5^+	M+1	131.6
	$C_2H_5^+$	M+29	162.6
	$C_3H_5^+$	M+41	–
Isobutane	$C_4H_9^+$	M+1	195.9
Ammonia	NH_4^+	M+1	204.0
		M+18	–

Consider the EI (70-eV) mass spectrum of amphetamine shown in Figure 1. The molecule fragmentation is quite complete in EI, thus producing very little molecular ion (M^+, 135 *m/z*); mass-to-charge ratios of the primary fragments are 44 and 91. These fragments are not unique and, in complicated samples, may not allow confident confirmation of the presence of the compound. Utilizing PCI with methane as the reagent gas produces protonated amphetamine $(M+H)^+$ at 136 *m/z*. Applying the more gentle ammonia reagent gas acts to further lower the degree of fragmentation and so $(M+H)^+$ dominates the spectrum. This selectivity characterizes chemical ionization and applies not only to the analyte of interest but also to the typical background interferences as well. For example, the typical hydrocarbons and related biogenic interferences are not ionized well by ammonia so an enhancement in signal is possible as well as a decrease in noise, which can combine to produce better results than obtainable with electron ionization (refer to Figure 2).

Adduct formation produces ions with mass-to-charge ratios larger than the molecular weight of the analyte molecule (M). For example, methane usually produces not only M+1 *m/z* as $(M+H)^+$, but M+29 *m/z* from the formation of $(M+C_2H_5)^+$ and M+41 *m/z* from the formation of $(M+C_3H_5)^+$. The conditions in the source (e.g., temperature, pressure, reagent gas) determine the relative ratios of the adducts produced. This is

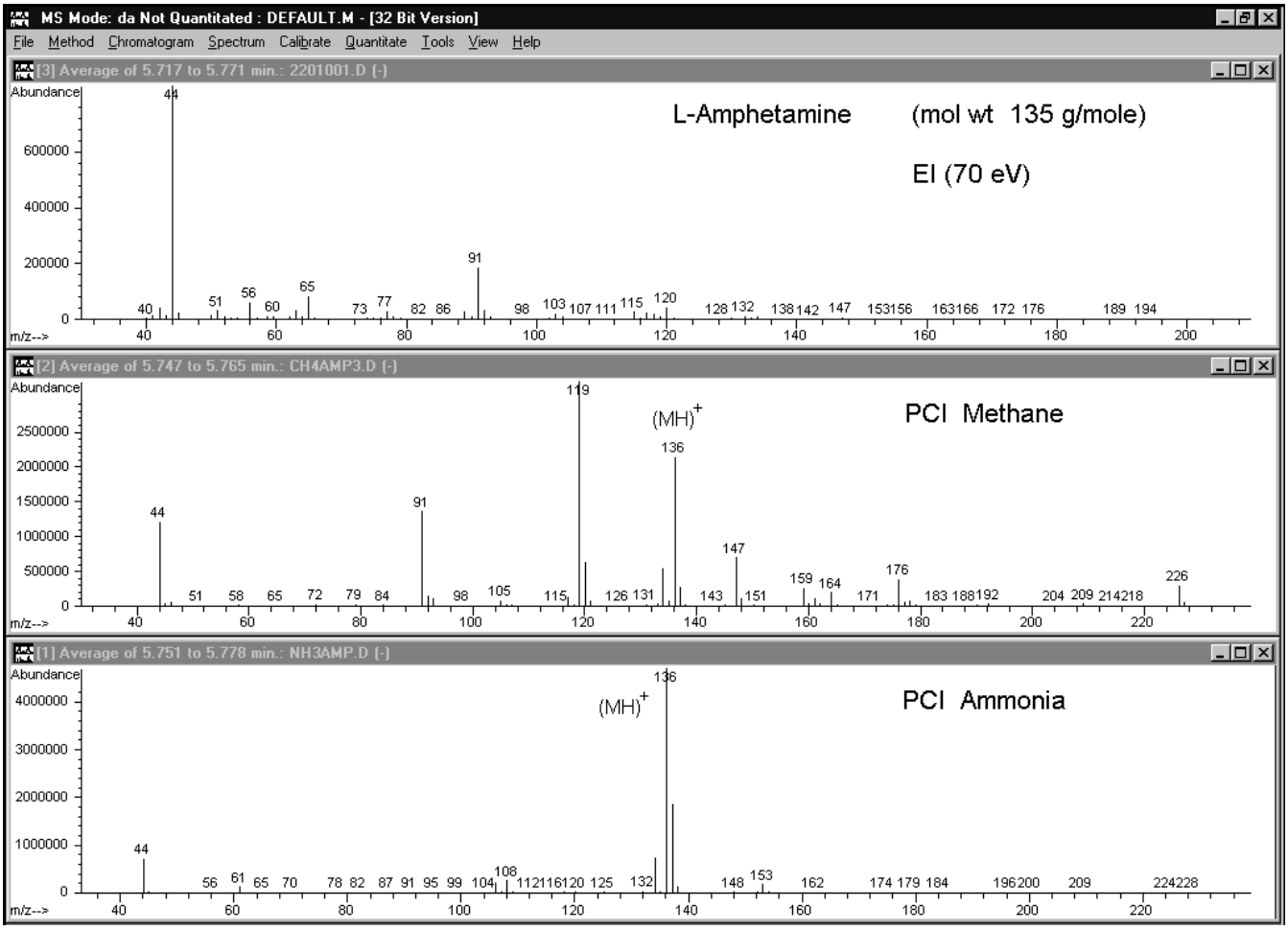
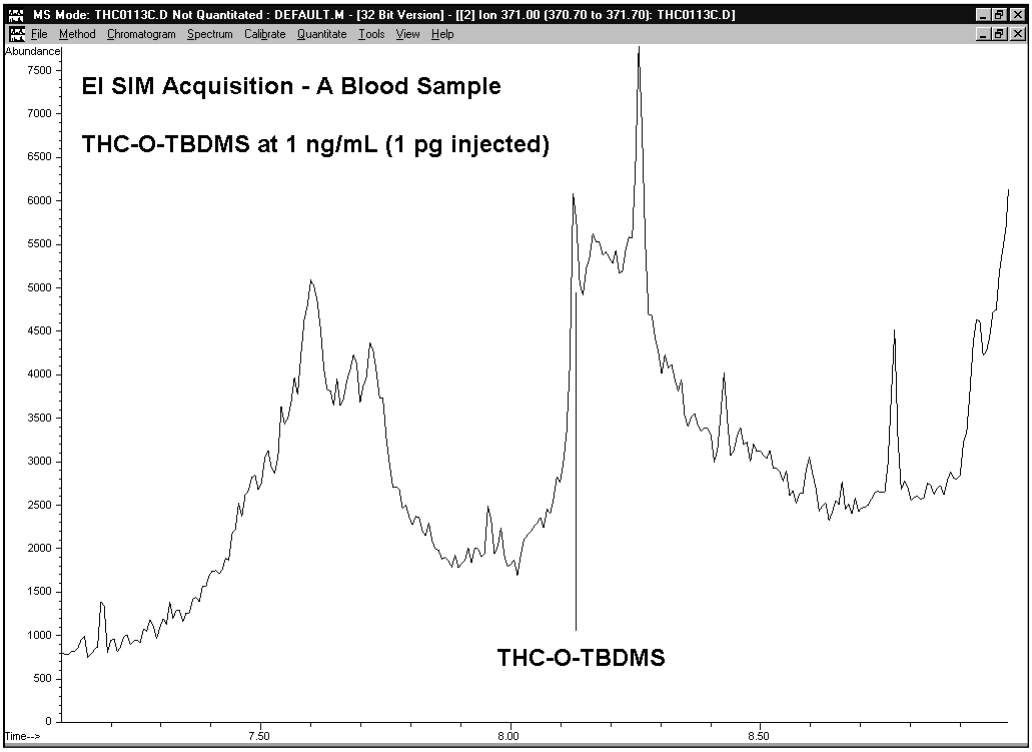


Figure 1. Mass spectrum of amphetamine (molecular weight 135 g/mole) as obtained by EI at 70-eV (upper panel), and PCI with methane (middle panel) and with ammonia (lower panel) reagent gas. Notice the decreasing degree of fragmentation most clearly indicated by the decreasing abundance of the 44 *m/z* fragment.

useful in two regards. First, if one is interested in determining the molecular weight of a compound, then adducts should appear in addition to M+1 to confirm the molecular weight assignment. To a certain extent, the ratios of the adducts can be manipulated by the source conditions and made more favorable for quantitation. An example of exploiting this characteristic is presented in detail in an Application Note on N-nitrosodimethylamine.[2]

There are other important ionization processes in PCI such as hydride extraction and charge exchange; more information is available in the *5973Network MSD Hardware Manual* and elsewhere.[3]

Because all compounds can be ionized by positive chemical ionization, a large number of PCI methods have been developed for a wide variety of compounds—from drugs in biological matrices to trace organic contaminants in environmental media. Selectivity is achieved by the proper choice of reagent gas and is an important approach to reducing interferences to analyte detection in complicated samples. Compounds that are prone to a high degree of EI fragmentation should be considered for PCI, especially when they are being analyzed and quantitated in complex matrices.



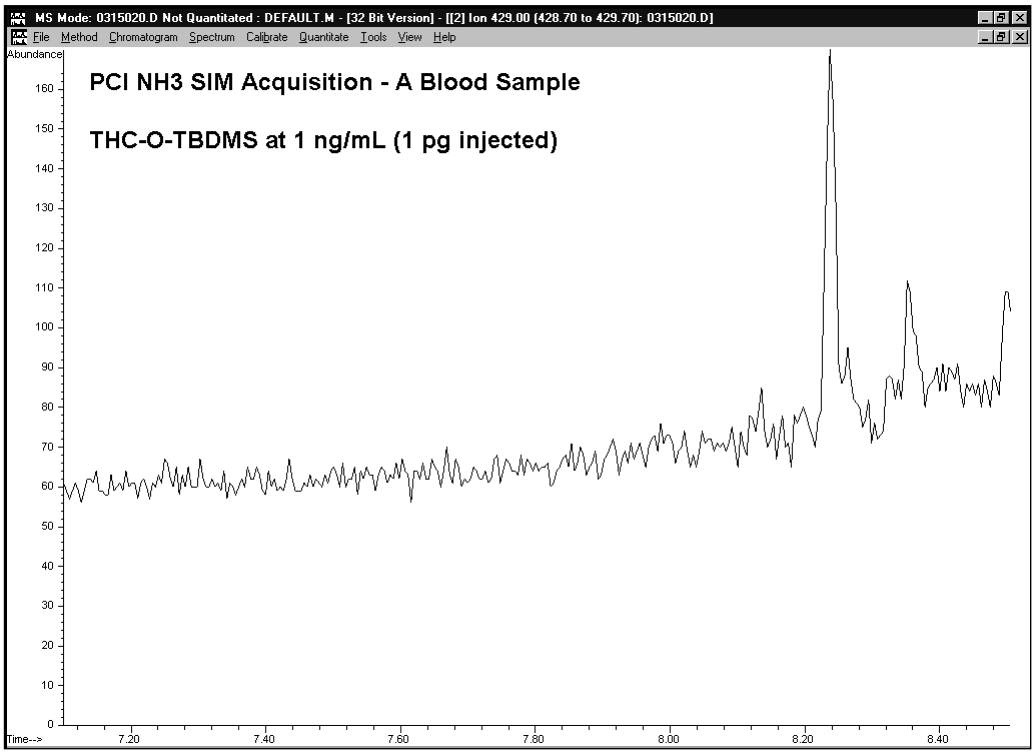


Figure 2. GC-MS SIM ion chromatograms acquired in EI and PCI for derivatized THC in blood at 1 ng/ml.[1] Notice that the EI chromatogram (upper panel) shows a more complex baseline than the PCI-ammonia chromatogram (lower panel) for their respective quantitation ions. The greatly reduced noise for the selected ion is characteristic of CI selectivity.

Electron Capture Negative Ion Chemical Ionization

Electron-capture negative ion (ECNI) chemical ionization, the second approach to CI available on the 5973N MSD platforms, mimics the electron-capture detector which has been so successfully used for trace analysis of organochlorine pesticides in complex environmental matrices. However the additional dimension of mass spectrometric information is provided by ECNI. In ECNI the "fast" electrons emitted by the filament are "thermalized", or slowed, by collisions with a buffer gas in the source (most commonly methane). These thermalized electrons form a "cloud" inside the source that allows electrons to be captured by electrophilic analyte molecules to form M^- or other negatively charged fragments or both. An example of the simplification in spectra produced by ECNI versus EI is given in Figures 3 and 4.

Compounds that are suitable for analysis by ECNI have electron affinities greater than 0.5-eV. These are typically compounds that contain electronegative groups such as oxygen, nitrogen, sulfur, phosphorus or the halogens, or extensive π systems (e.g., polycyclic heteronuclear hydrocarbons). Most synthetic organic chemicals and most biologically interesting compounds contain electronegative elements, or can be made to include electrophilic constituents by derivatization. As a result, a wide range of compounds is amenable to ECNI analysis, such as many of the persistent organochlorine contaminants and endocrine disrupters of environmental concern,[4] and drugs of abuse in complex media (e.g., cannabinoids in blood[5] or a "date rape drug" in hair and urine).[6]

Operating Features of the 5973N MSD Series in Chemical Ionization

The 5973N Series MSDs make chemical ionization operation nearly as easy as standard EI operation. The autotuning algorithm performs an autotune in ECNI and PCI with methane reagent. Autotunes are also performed with isobutane and ammonia in ECNI. Tuning also supports isobutane and ammonia in PCI, with options for other user-designated reagent gases.

A clean gas manifold allows selection of either of two reagent gases with fine control of gas delivery to the source. This fine control is important in PCI for generating the desired adduct ratios and maintaining them. An example of the high precision available in PCI operation is presented in Table 2, which shows the reproducibility of the response for the quantitation ion and the reproducibility of the ratio of the quantitation ion to the confirming ion for N-nitrosodimethylamine at 20 fg/µl. Notice that even at this extremely low concentration the ion responses are very reproducible.

Table 2. PCI reproducibility in SIM acquisition with ammonia reagent gas for N-nitrosodimethylamine (NDMA). Precision is indicated by the following: the ratio of protonated NDMA, $[NDMA+H]^+$ = 75 *m/z* to ammonium adduct, $[NDMA+NH_4]$ = 92 *m/z,* as the ratio of the integrated areas of 75 *m/z* : 92 *m/z* ions; and the precision of response of the 92 *m/z* quantitation ion for five replicate 50-µl injections at five concentrations. Notice the precision in both response and the ratio of responses is, on average, better than 3% even at very low concentrations demonstrating the integrity of the entire 6890/5973N system.

Concentration as fg NDMA / µl	RSD Ratio 75 *m/z* / 92 *m/z*	RSD Response by 92 *m/z* area
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

In short, the 5973N MSD has been designed for ease of operation in PCI and ECNI while producing superior spectra and sensitivity with outstanding reproducibility. The principal advantages of using CI ionization on the 5973N MSDs include "user-friendly" operation (automated tuning), high sensitivity, classical spectra, reproducible ion ratios, and stable and reproducible SIM ion ratios.

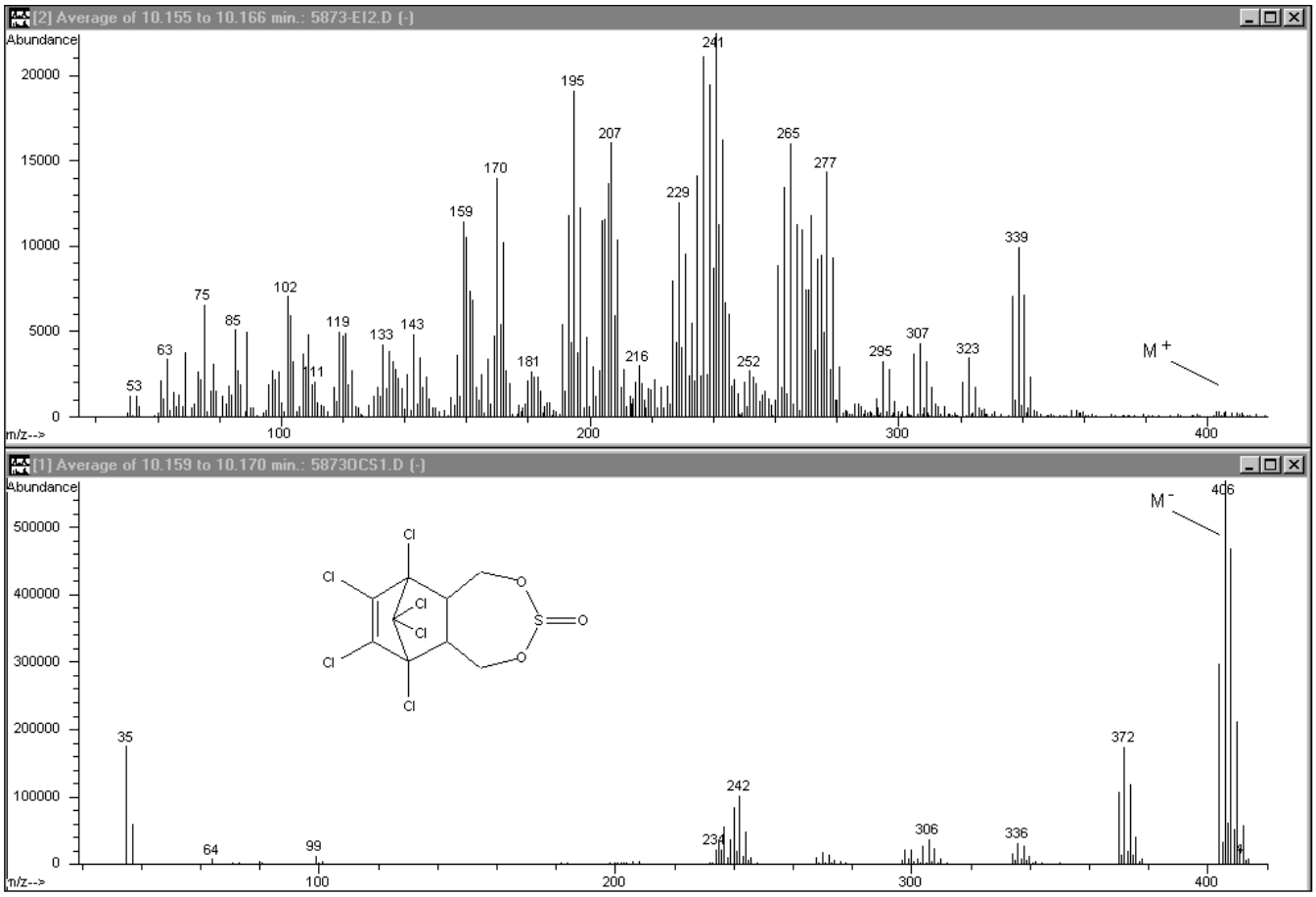


Figure 3. Mass spectrum of endosulfan ($C_9H_6Cl_6O_3S$, m. wt. 406 g/mole, CAS number 115-29-7) in EI (upper panel) and ECNI (lower panel). EI produces extensive fragmentation with very little molecular ion formed (M^+), but the molecular anion (M^-) is the most abundant peak in ECNI. The fragment at 372 *m/z* is formed by loss of chlorine which also appears at 35 and 37 *m/z*.

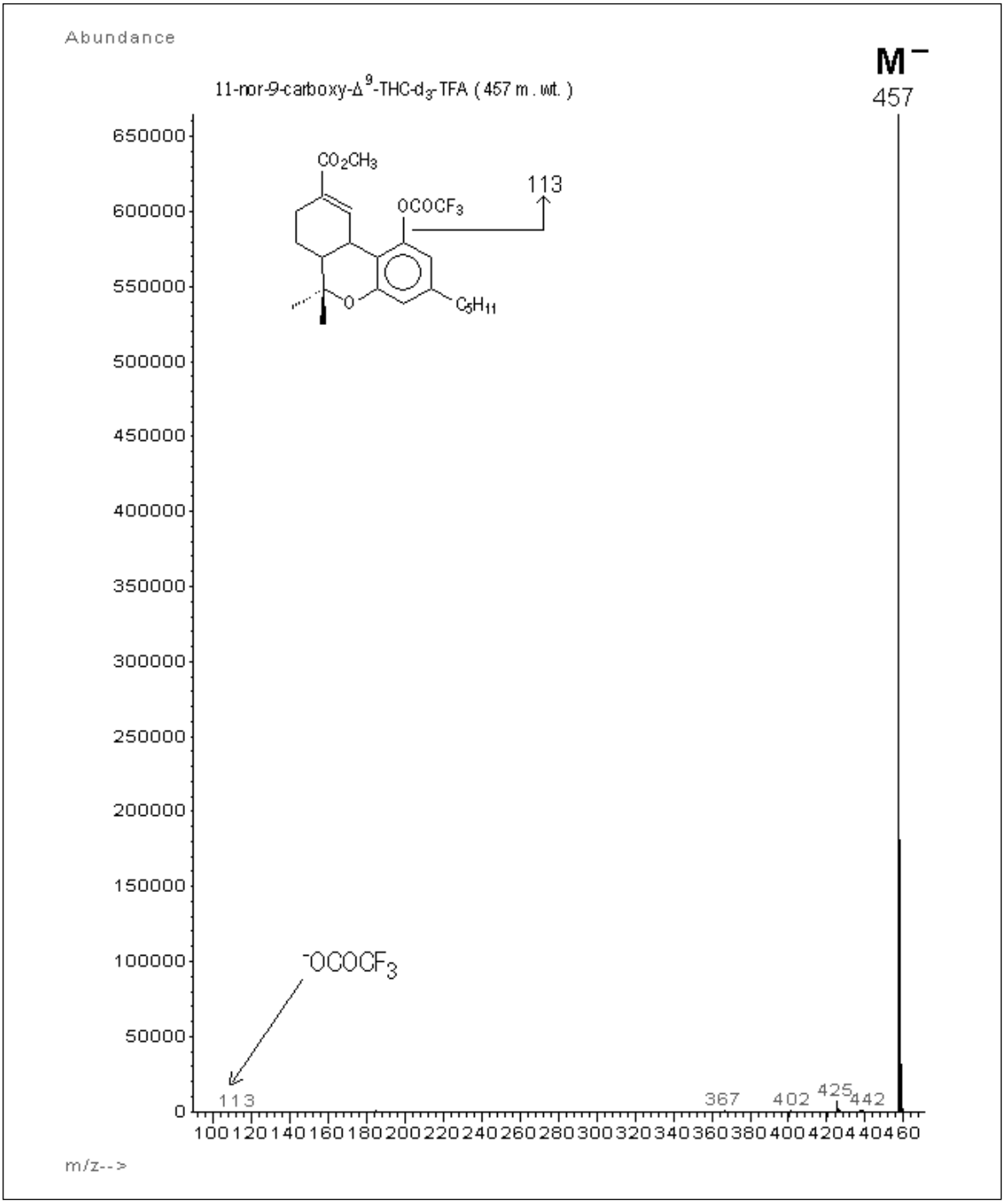


Figure 4. ECNI mass spectrum of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-d_3 the deuterated surrogate for the major metabolite of Δ^9-tetrahydrocannabinol (THC) found in marijuana, as the trifluoroacetyl derivative. The mass spectrum consists almost exclusively of the molecular ion M^- at *m/z* 457; it shows an undetectable amount of *m/z* 113 ion in the spectrum which reflects the inertness of the 5973N ECNI source. Source activity increases the *dissociation* of derivatized compounds (which in this case would appear as the presence of $OCOCF_3$ anion) and thereby decreases sensitivity.

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