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Isotopic Analog as the Internal Standard for Quantitative Determination: Evaluation of Mass Spectra of Commonly Abused Drugs and Their Deuterated Analogs

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ABSTRACT: Mass spectra of several commonly abused drugs and their deuterated analogs are compared and evaluated with emphasis on the selection of suitable ions for selective ion monitoring when the isotopic analogs are used as the internal standards in a quantitative determination process. Ions selected for this purpose should be of relative high mass with significant intensities, retain at least three labeling isotopes, and be free of interference from the corresponding compound.

KEYWORDS: toxicology, abuse drugs, internal standard, analogs, chemical analysis, amphetamine, methamphetamine, cocaine, benzoylecgonine, methadone, codeine, morphine, phencyclidine, 9-carboxy-11-nor-delta-9-tetrahydrocannabinol

Internal standard method is considered [*1*] the most effective approach in a quantitative analysis process. Among various types [*2*] of suitable internal standard candidates, a stable isotope-labeled analog of the analyte is most often used. Selective monitoring of corresponding ions [*3,4*] generated by the analyte and the isotopic internal standard, followed by the evaluation on the ion intensity ratios in the calibration standard and in the test sample, provide the basis for a quantitative gas chromatographic/mass spectrometric (GC/MS) analytical process [*5*].

Before a specific isotopic analog can be adopted as an internal standard in a GC/MS application, factors such as mass difference, isotopic purity, and mass spectrometric fragmentation characteristics have to be evaluated. In this study, the mass spectra of several commonly abused drugs along with their isotopic analogs are presented; these spectrometric data are then critically evaluated and ions suitable for selected ion monitoring (SIM) in GC/MS applications are identified.

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Materials and Methods

The sources of drugs and their isotopic analogs used in this study are listed in Table 1. They were used as supplied for solution preparation and derivatization, if needed, without further purification. Since these spectra were obtained under GC/MS (electron impact [EI]) conditions, drug purity is not considered a critical factor.

A Hewlett-Packard 5970B mass selective detector coupled to a 5890 series Hewlett-Packard gas chromatograph equipped with a 15-m by 0.251-mm inside diameter (ID) (0.25-μm film thickness) J & W DB-5 column (Folsom, California) is used for analysis. The injector temperature is maintained at 270°C, and the collision energy is 70 eV. The mass analyzer was scanned from *m/z* 45 to a mass unit higher than the molecular weight of the compound under examination.

Results and Discussion

Criteria for Selecting an Isotopic Analog as the Internal Standard

The main advantage of using an isotopic analog of an analyte as the internal standard is that their similarities in chemical properties and the mass spectrometric fragmentation

TABLE 1—*Compound sources and derivatization.*

Compound	Source[a]	Derivatization
Amphetamine HCl	Alltech	trichloroacetic anhydride
1-Phenyl-d_5-aminopropane	Radian	same as above
Methamphetamine HCl	Sigma	same as above
1-Phenyl-2[methyl-d_3-amino]-propane-1,2-d_2	Sigma	same as above
Benzoylecgonine	Radian	iodopropane
[*N*-Methyl-d_3]benzoylecgonine	RTI	same as above
Cocaine (from benzoylecgonine)	Radian	iodomethane
[*N*-Methyl-d_3]cocaine (from [*N*-methyl-d_3]benzoylecgonine)	RTI	same as above
Methadone	RTI	none
Methadone-1,1,1-d_3 HCl	RTI	none
Codeine	Radian	acetic anhydride
[*N*-Methyl-d_3]codeine	RTI	same as above
Morphine	Sigma	same as above
[*N*-Methyl-d_3]morphine	RTI	same as above
Phencyclidine	Sigma	none
Phencyclidine-d_5	Sigma	none
9-Carboxy-11-nor-delta-9-tetra-hydrocannabinol (THC Acid)	RTI	iodomethane
9-Carboxy-11-nor-delta-9-tetra-hydrocannabinol-5′-d_3	RTI	same as above

[a]Alltech = Alltech-Applied Science, State College, PA; Radian = Radian Corporation, Austin, TX; Sigma = Sigma Chemical Company, St. Louis, MO; RTI = Research Triangle Institute, Research Triangle Park, NC.

process compensate for possible errors that may derive from the loss of the analyte in the sample preparation process or the differential gas chromatographic and mass spectrometric characteristics. However, there are several important parameters that require careful consideration when an isotopic analog is adopted as the internal standard.

First, the isotopic analog should be labeled with a sufficient number of a selected isotope so that the corresponding ions selected from the internal standard and the analyte will have a significant difference in their masses. If the difference is not sufficient, the $[M + n]$ ion (in the analyte) due to the naturally occurring isotope abundance may make a significant contribution to the intensity of the ion (in the isotopic analog internal standard) that corresponds to the $[M]$ ion of the analyte. (M is the mass of the ion derived from the analyte selected for monitoring, and n is the nominal mass difference of the ions selected from the analyte and the internal standard.) If deuterium, as in most realistic cases, is used as the labeling isotope for the internal standard, a difference in three mass units between the analyte and the internal standard is considered sufficient under normal circumstances. However, if the concentration of the analyte is unproportionally higher than the concentration of the internal standard used, the intensity of the $[M + 3]$ ion originated from the analyte may become significant enough to require an additional analysis on a diluted aliquot.

Second, the isotopic analog should be manufactured with sufficient isotopic purity. Otherwise, the addition of the internal standard may result in the observation of a significant amount of the analyte in a true negative sample and may also introduce errors in quantification. This will become a problem of concern, especially when a high concentration of the internal standard is used. This problem has been well addressed elsewhere [5].

Finally, the analyte and the isotopic analog should undergo an appropriate fragmentation process to generate several high intensity ions that include the labeling isotopes with insignificant $[M - n\text{H}]$ ions. In other words, the labeling isotopes must be positioned at appropriate locations in the molecular framework of the compounds so that, after the fragmentation process, sufficient number of high-mass ions that retain the labeling isotopes are present with significant intensities and will not contribute to the intensities of the corresponding ions derived from the analyte. These ions and their counterparts in the analyte may then be monitored for ion ratio evaluation to facilitate qualitative compound identifications and quantitative determinations. The main thrust of this paper is precisely on this matter. Empirical mass spectrometric data of several drugs and their isotopic analogs are evaluated with emphases on examining the suitability of these intended isotopic internal standards and selecting appropriate ions for monitoring.

Comparison and Evaluation on Mass Spectrometric Data of Commonly Abused Drugs and Their Isotopic Analogs

Along with the mass spectra presented in Figs. 1 through 8, corresponding ions from the analytes and their isotopic analogs that retain the labeling isotopes are presented in Table 2. With the exception of methadone, all ion intensities are normalized to the most intense ions in their respective spectra. In general, only one ion in a cluster of ions is selected; ions resulting from natural occurring isotopes or the loss of nH atoms are not listed. With the exception of methadone, ions with intensities less than 10% of the most intense ion within the scanned range (m/z 45 to the molecular weight) are not listed. Not all ions that retain the labeling isotopes are suitable for monitoring. One of the most common problems is the appearance of an ion with an identical nominal mass that will interfere with the ion-intensity measurement of the corresponding ion in the counter compound. These situations are footnoted as *c*, *d*, and *e* in Table 2. For this purpose,

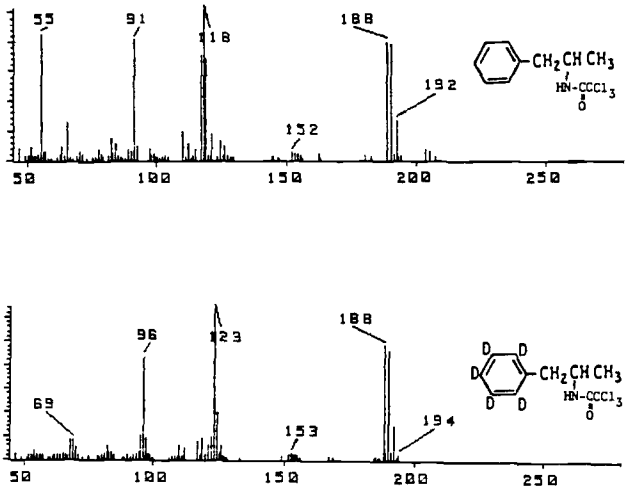

FIG. 1—*Mass spectra of trichloroacetyl derivative of amphetamine* (top) *and its deuterated analog* (bottom).

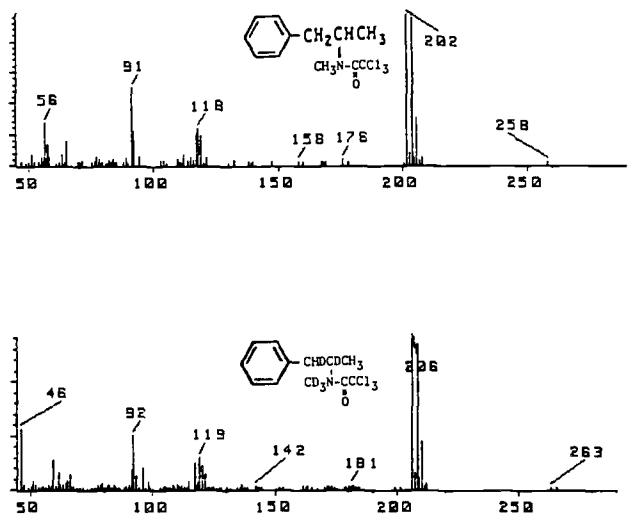

FIG. 2—*Mass spectra of trichloroacetyl derivative of methamphetamine* (top) *and its deuterated analog* (bottom).

the appearance of a 5% intensity (relative to the intensity of the ion with the same mass in the counter compound) is considered significant and noted.

Amphetamine—Among the three ions with significant intensities that retain the labeling deuterium atoms, only the *m/z* 118/123 and 91/96 ion pairs are suitable for monitoring. Significant intensities of *m/z* 112 and 117 ions are observed in the spectra of the isotopic analog and the analyte, respectively, causing interference. Thus, this deuterated amphetamine is not a suitable internal standard if more than two ions are required for ion-ratio evaluation and quantification.

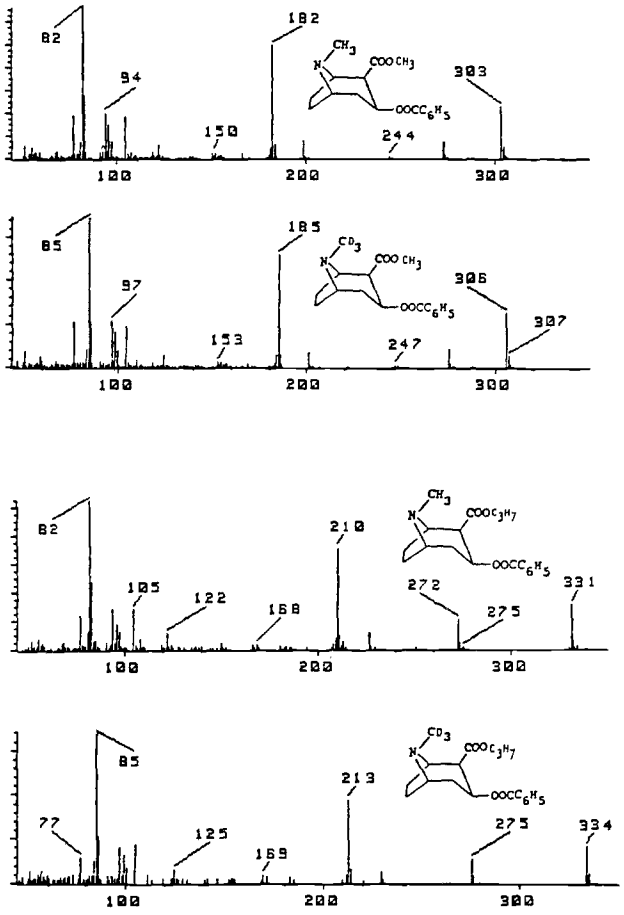

FIG. 3—*Mass spectra of cocaine* (top), *deuterated cocaine* (upper middle), *propyl ester of benzoylecgonine* (lower middle), *and propyl ester of deuterated benzoylecgonine* (bottom).

Methamphetamine—The first three ion pairs listed in Table 2 represent the natural occurring isotope abundance of the three chlorine atoms introduced in the derivatization process. The chlorine isotope distribution pattern prohibits the use of the *m/z* 202/206 and 206/210 ion pairs. If the *m/z* 204/208 ion pair is to be used, one should be aware of the fact that the three chlorine atoms in the analyte derivative contribute substantially to the *m/z* 208 ion intensity. The only other ion pair that may be of apparent value is *m/z* 56/59 representing the retention of three deuterium atoms in this fragment. However, the low mass nature of this pair makes it a poor choice.

Cocaine and Benzoylecgonine (Propyl Ester)—Since cocaine and the propyl ester of benzoylecgonine differ only by a ethylene (C_2H_4) group in the alkyl group, they are presented together (Fig. 3) for comparison. Ions derived from these two compounds containing this differentiating group will have a difference of 28 AMU, as seen in the first four pairs of ions listed in the table; those fragments without this group will have identical mass. Five pairs of ions are available for monitoring for both compounds. However, the 82/85 pair may not be desirable due to the relatively low mass nature.

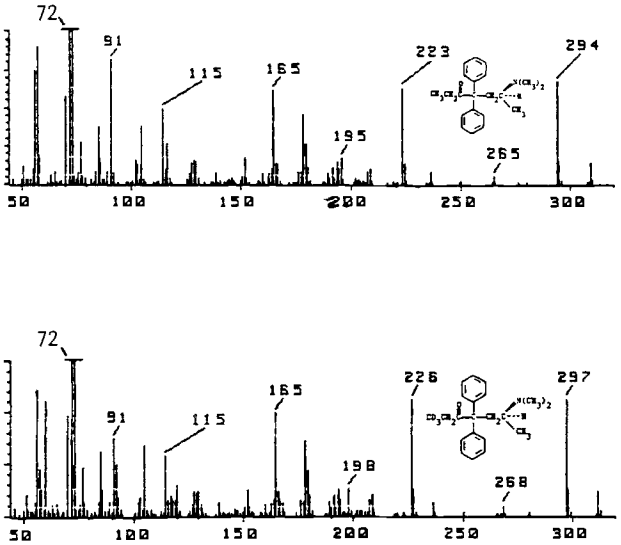

FIG. 4—*Mass spectra of methadone* (top) *and its deuterated analog* (bottom).

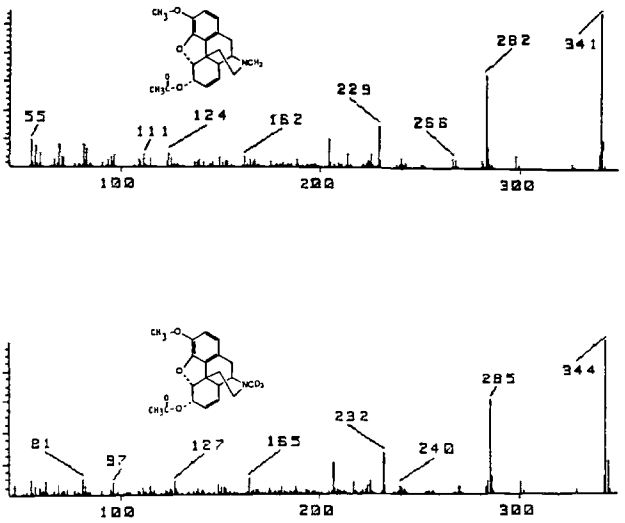

FIG. 5—*Mass spectra of acetylcodeine* (top) *and its deuterated analog* (bottom).

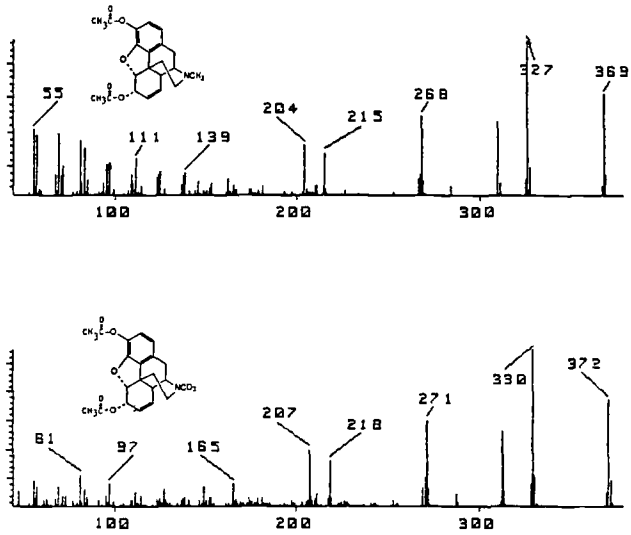

FIG. 6—*Mass spectra of acetylmorphine* (top) *and its deuterated analog* (bottom).

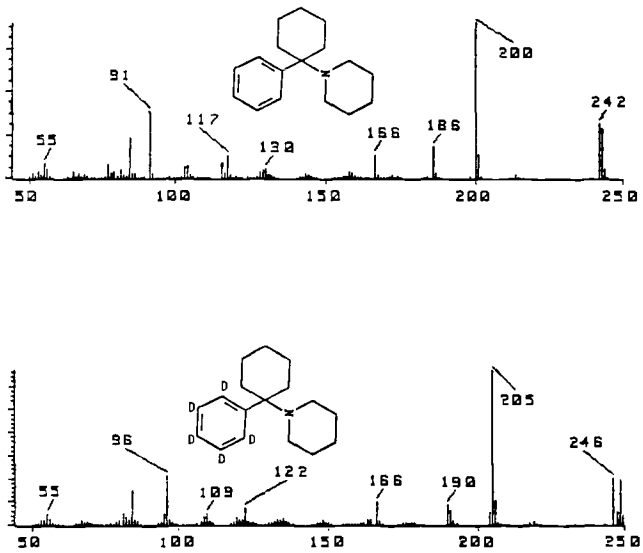

FIG. 7—*Mass spectra of phencyclidine* (top) *and its deuterated analog* (bottom).

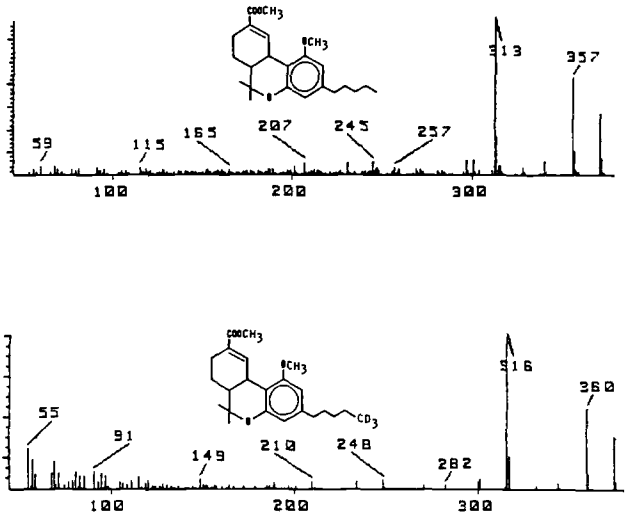

FIG. 8—*Mass spectra of methyl derivative of 9-carboxyl-11-nor-delta-9-tetrahydrocannabinol* (top) *and its deuterated analog* (bottom).

TABLE 2—*Corresponding ions*[a] *in analyte and isotopic analog.*

Compound	Molecular Weight[b]	Analyte Ion	Isotopic Analog Ion
Amphetamine (trichloroacetyl Der.)	267	118 (100)	123 (100)
		112 (11)[c]	117 (12)[c]
		91 (79)	96 (68)
Methamphetamine (trichloroacetyl Der.)	281	206 (31)[d]	210 (32)[d]
		204 (97)	208 (97)
		202 (100)[e]	206 (100)[e]
		119 (19)[c]	121 (16)[c]
		118 (25)[d]	120 (16)[d]
		117 (21)[c]	119 (22)[c]
		91 (51)[c]	92 (36)[c]
		65 (15)[d]	66 (10)[d]
		57 (13)[c]	61 (10)[c]
		56 (28)	59 (20)
Cocaine	303	303 (35)	306 (35)
		272 (11)	275 (11)
		198 (12)	201 (9)
		182 (76)	185 (75)
		97 (10)[d]	100 (10)[d]
		96 (22)[d]	99 (23)[d]
		94 (30)[e]	97 (31)[e]
		82 (100)	85 (100)
Benzoylecgonine (propyl der.)	331	331 (30)	334 (24)
		272 (19)	275 (15)
		226 (10)	229 (7)
		210 (68)	213 (56)
		122 (10)[d]	125 (9)[d]
		97 (12)[d]	100 (10)[d]
		96 (16)[d]	99 (19)[d]
		94 (27)[e]	97 (23)[e]
		82 (100)	85 (100)

TABLE 2—*Continued.*

Compound	Molecular Weight[b]	Analyte Ion	Isotopic Analog Ion
Methadone	309	309 (21)	312 (20)
		294 (100)	297 (100)
		223 (97)	226 (100)
		195 (25)[d]	198 (23)[d]
Codeine (acetyl der.)	341	341 (100)	344 (100)
		298 (8)	301 (8)
		282 (60)	285 (61)
		229 (27)	232 (26)
		204 (19)[c]	207 (20)[c]
Morphine (acetyl der.)	369	369 (67)	372 (68)
		327 (100)	330 (100)
		310 (49)	313 (48)
		268 (53)	271 (55)
		215 (29)	218 (29)
		204 (35)[e]	207 (36)[e]
		146 (12)[c]	149 (12)[c]
Phencyclidine	243	243 (35)	248 (29)
		242 (35)	246 (29)
		200 (100)	205 (100)
		186 (20)	190 (13)
		117 (15)[d]	122 (11)[d]
		91 (43)	96 (31)
THC Acid (methyl der.)	372	372 (40)	375 (38)
		357 (63)	360 (56)
		341 (8)	344 (9)
		313 (100)	316 (100)
		297 (9)[c]	300 (10)[c]
		245 (8)[c]	248 (10)[c]
		207 (8)[c]	210 (10)[c]

[a]Ions in each compound are listed in the order of decreasing mass. Numbers inside parentheses are relative intensities. Intensities of ions are normalized to *m/z* 294 and 297 for methadone and its isotopic analog. For other compounds, relative intensities are normalized to the most intense ion within the mass range scanned for the respective compound.

[b]Nominal molecular weights of the analytes, not their isotopic analogs, are listed in this column.

[c]These ion pairs are not suitable for monitoring due to the appearance of ions in the analyte and the isotopic analog, which will interfere with each other.

[d]Spectra from the isotopic analog appear to contain ions with significant intensities that will interfere with the corresponding ions in the analyte.

[e]Spectra from the analyte appear to contain ions with significant intensities that will interfere with the corresponding ions in the isotopic analog.

Methadone—There is only one ion, *m/z* 72, with significant intensity in both the spectra of methadone and its isotopic analog. To facilitate data evaluation, ion intensities are normalized to the *m/z* 294 and 297 ions, respectively. Although there are four ions that retain the labeling isotopes in the spectrum of the isotopic analog, the intensities of these ions are very low (about 3% of the *m/z* 72 ion). Thus, using the proposed isotopic analog as the internal standard will require the monitoring of these low-intensity ions, with consequent loss in assay sensitivity.

Codeine and Morphine (Acetyl Derivative)—Ion pairs that are suitable for codeine determination are *m/z* 344/341, 282/285, and 229/232; for morphine determination *m/z* 369/372, 310/313, 327/330, 268/271, and 215/218. The first two ion pairs in these two

compounds differ by 28 amu, reflecting the presence of a methoxyl group (in codeine) and an acetyl group (in morphine) in these ions and the preservation of similar fragmentation pathways in these two compounds.

Phencyclidine—Several pairs of ions which meet the selection criteria are available in this pairs of compounds. It should be noted that the *m/z* 242/246 and 186/190 pairs differ by four mass units, indicating the loss of one labeling deuterium atom from the isotopic analog and the corresponding hydrogen atom from the analyte.

9-Carboxyl-11-nor-delta-9-tetrahydrocannabinol—Although there are many pairs of ions listed in Table 2, only three pairs are suitable for the intended use: *m/z* 372/375, 357/360, and 313/316. The low intensities of the other ions and the apparent appearance of interfering ions in the counter compounds render the use of other ions impractical.

Conclusion

The comparison and evaluation of spectra obtained from the analytes and their isotopic analogs demonstrate that the selection of an isotopic analog as an internal standard is not a trivial matter. Before an isotopic analog is synthesized, the mass spectrometric fragmentation pattern of the analyte should be understood; thus, desired isotopes may be incorporated into appropriate positions in the framework of the molecule. A valuable isotopic analog should generate sufficient number and significant intensity of ions that retain the labeling isotopes in the fragmentation process. For compounds that require derivatization before GC/MS analysis, the selection of an appropriate derivatizing reagent is also important.

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