TECHNICAL NOTE

Josh B. Steeves,[1] B.S.; Havaleh M. Gagné,[1] B.S.; and Eric Buel,[1] Ph.D.

Normalization of Residual Ions After Removal of the Base Peak in Electron Impact Mass Spectrometry

REFERENCE: Steeves JB, Gagné HM, Buel E. Normalization of residual ions after removal of the base peak in electron impact mass spectrometry. J Forensic Sci 2000;45(4):882–885.

ABSTRACT: The mass spectra of compounds that produce limited detail under electron impact conditions may yield useful data for identification purposes when further examined. Through the mathematical removal of the base peak, previously noninformative ions become discriminating and useful for identification. In this work we show that this process of base peak removal and the re-normalizing of the remaining ions is reproducible under a variety of conditions and can be valuable for compound identification.

KEYWORDS: forensic science, illicit drug identification, gas chromatography-mass spectrometry, amphetamine, methamphetamine

Gas chromatography tandem to mass spectrometry is the method of choice for the routine analysis of drugs of abuse within the forensic arena. The combination of these two techniques allows the separation of various drugs with their subsequent identification by mass spectrometry. Most drugs of abuse are amenable to this method of analysis. However, a number of drugs yield mass spectra that are not immediately determinative. Amphetamines and related compounds have mass spectra predominated by a single ion, with limited additional ions at very low abundance. Identification of such compounds with this type of mass spectrum usually requires synthesis of derivatives of the compound (1,2), or utilization of other methods such as chemical ionization (3), infrared spectroscopy, or nuclear magnetic resonance (4). These methods can be time-consuming or require additional instrumentation.

We have examined the ions that are significantly less abundant than the base peak for amphetamine and related compounds to determine if these ions may be of value. If the base peak is removed, and the resulting spectrum is normalized using the peak of next greatest abundance, identification becomes possible with the existing spectrum. The ions "beneath" the predominate base peak are consistently present in the spectrum of a particular compound and do not reflect instrumental noise. This report shows how this technique can be used to identify even the closely related compounds methamphetamine and phentermine.

Materials and Methods

Sample Preparation

The drug standards used for this study were obtained from Sigma, St. Louis, MO, or Radian, Austin, TX, as solutions with a concentration of 100 μg/mL. These were diluted with ethanol prior to GC/MS analysis (10 to 20 μL of standard diluted to 125 μL). See Table 1 for a listing of the drugs examined.

GC/MS Analysis

Samples were analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a Model 5970 Mass Selective Detector, (Hewlett Packard, Palo Alto, CA). Hewlett Packard Chemstation Software, version A.02.00, was used to collect and manipulate the data. The instrumental conditions were as follows:

Column: 20 m by 0.18 mm, DB-5
Injector temperature: 250°C
Transfer line temperature: 280°C
Temperature program: 60°C for the first minute, then 15°C/min to 300°C
MS solvent delay 2 min, total run time 20 min
Carrier gas: helium at 25 psi
Ion range: 30 to 400 amu

Retention time consistency was established by adding octadecane to each sample and converting all retention times into relative retention times by dividing the retention time of the compound of interest by the octadecane retention time.

Data Analysis

For each spectrum, the predominate ion (base peak) was removed using the Chemstation software "msclip" command. The resulting spectrum was normalized to an abundance of 10,000 for the next highest ion. The abundance values for each spectrum were collected in table format. The final values for comparison were calculated as abundance percentages of the ion of interest to the new predominate ion peak.

[1] Vermont Forensic Laboratory, Waterbury, VT 05676.

Received 23 Aug. 1999; and in revised form 8 Oct., 22 Oct. 1999; accepted 25 Oct. 1999.

TABLE 1—*Compounds examined.*

Amphetamine	Phenylpropanolamine
Chloramphetamine	3,4-Methylenedioxyamphetamine (MDA)
Phentermine	Methamphetamine
Ephedrine	Pseudoephedrine
3,4-Methylenedioxymethamphetamine (MDMA)	Diphenhydramine
Psilocin	Dextromethorphan
Propoxyphene	Amitriptyline
Doxepin	Chlorpromazine

Results and Discussion

The mass spectrum in conjunction with the GC retention time routinely provides enough information to identify an unknown drug. In those instances where a mass spectrum of a drug is characterized by only a predominate ion, other analytical techniques may be employed to obtain an identification. These additional techniques may not be necessary since the spectra of these compounds often offer useful identifying information when the predominate ion is removed from the spectrum and the remaining ions normalized to that of the second highest ion in the original spectrum.

Figures 1*a* and *b* show an example of the original and normalized spectra of MDMA and pseudoephedrine. The structural backbones of these compounds are similar, giving rise to the predominate base peak apparent in both spectra.

These molecules have very different functional groups but these moieties add only limited mass spectral detail. However, when base peak removal and normalization of the spectra is employed, these structural differences become important features yielding detail in the mass spectrum as many additional ions become significant for use in identification.

Figure 2 shows the normalized spectra of drugs that contain a base ion m/z 58 and only limited additional structural information in the 5 to 10% abundance range in their normal mass spectra. This figure clearly shows how each of these compounds can be distinguished using the normalization procedure. Table 1 lists other drugs that have spectra with limited detail that have been examined in this study and are clearly distinguished using this technique.

Methamphetamine and phentermine are two drugs that yield similar original and normalized mass spectra and, under our instrumental conditions, have close GC retention times. Figure 3 shows the normalized mass spectra of methamphetamine and phentermine. The normalized spectra are considerably different from the spectra of the other compounds examined but are similar to each other. Both show a high abundance of ions m/z 91, 65, 59, and 39 with the residual abundance patterns of both appearing very similar. A close inspection reveals a number of ions present for both compounds that occur at considerably different abundances. Thirty-five replicate analyses were performed to see if these abundance differences were reproducible. Table 2 shows the average observed abundance, average deviation and the abundance ranges for selected ions for these two drugs.

Significant abundance differences between methamphetamine and phentermine are seen for the ions detailed in Table 2. The average deviation for the abundance in each ion is relatively small at no more than 4%. The observed abundance ranges do not overlap for these ions. The ion m/z 56 showed exceptional differences, with the average abundance for methamphetamine at 69% versus 12% for phentermine.

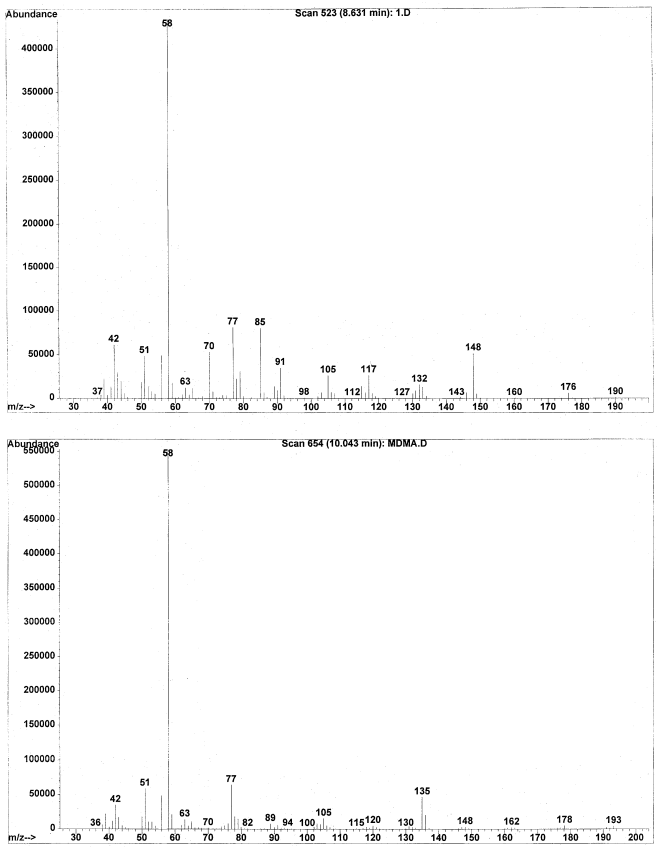

FIG. 1*a*—*Original spectra for pseudoephedrine and MDMA.*

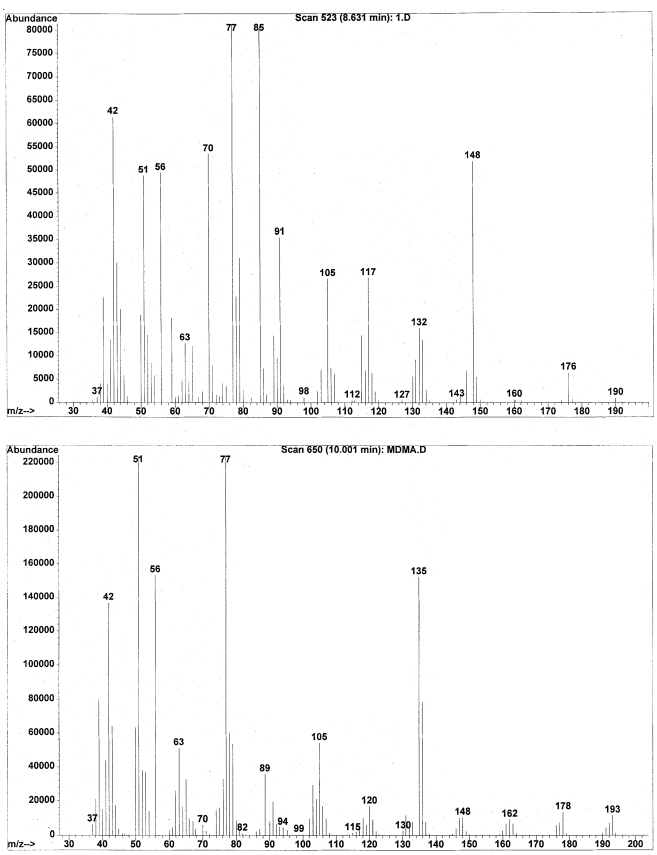

FIG. 1*b*—*Normalized spectra for pseudoephedrine and MDMA.*

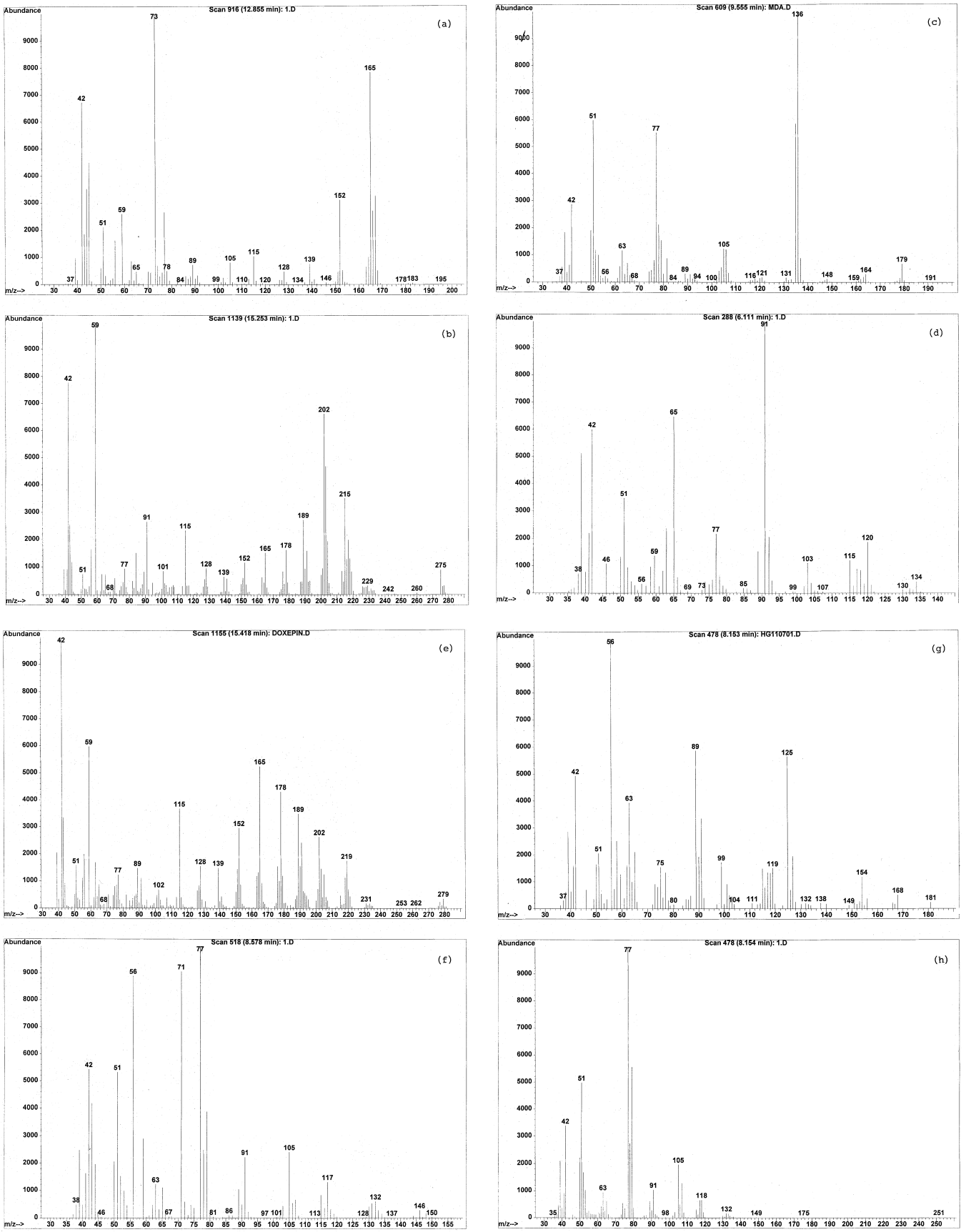

FIG. 2—*Normalized spectra for* (a) *diphenhydramine*, (b) *amitriptyline*, (c) *MDA*, (d) *amphetamine*, (e) *doxepin*, (f) *ephedrine*, (g) *chloramphetamine*, *and* (h) *phenylpropanolamine.*

TABLE 2—*Statistical data for selected ions for methamphetamine and phentermine.*

	Methamphetamine			Phentermine		
	Av. Value	Std. Deviation	Ranges	Av. Value	Std. Deviation	Ranges
Peak:						
41	29%	1.86%	25%–32%	75%	4.74%	64%–89%
42	59%	3.12%	53%–65%	80%	5.27%	67%–92%
56	69%	3.89%	57%–77%	12%	0.96%	9%–14%
134	21%	2.62%	13%–24%	44%	3.00%	38%–53%

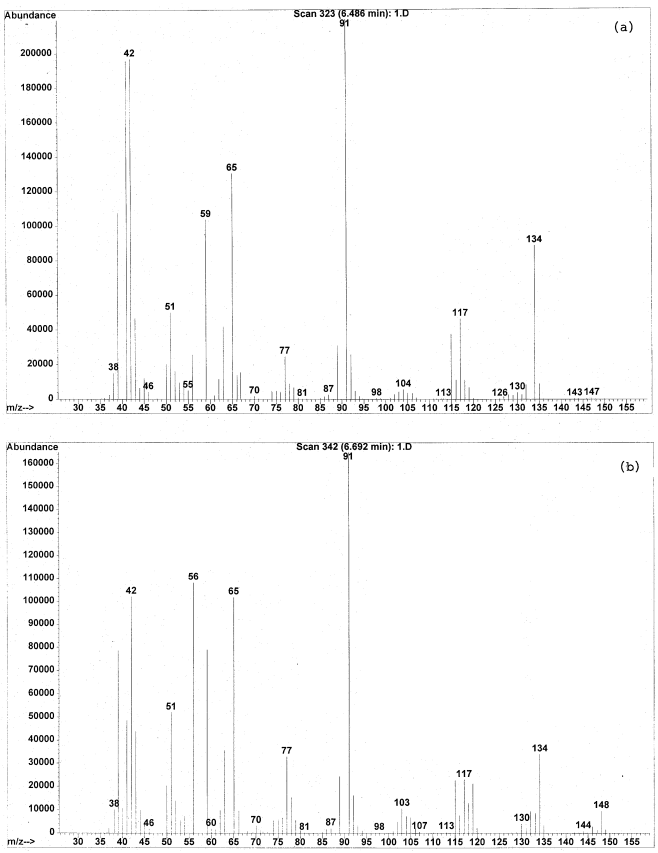

FIG. 3—*Normalized spectra for* (a) *phentermine,* (b) *methamphetamine.*

The m/z range of 115 to 119 provided another suitable means for the discrimination of methamphetamine from phentermine. We have found that all methamphetamine spectra displayed a "triplet" set of ions at m/z 115, 117, and 119 all at a similar abundance, while phentermine displayed a "doublet" set of ions at m/z 115 and 117. The 119 ion found in phentermine was always significantly lower in abundance than the 115 and 117 ions.

The normalization procedure was tested under conditions that could stress the system and may typify actual casework analysis. Drugs were analyzed at relatively high concentrations (0.31 to 0.5 mg/mL, compared to typical concentrations of 0.08 to 0.16 mg/mL used for the rest of the study) to determine if any fluctuations occurred in ions or abundances. The drugs evaluated in this study were tested over a period of several months in which many instrument autotunes were performed. Furthermore, the instrument received routine maintenance during this time. No noticeable change in ions or abundance values was observed when these modifications were made.

Alternative procedures to the normalization technique presented here could also be employed to expand the *y*-scale to reveal the residual ions. Using a fixed multiple, where the *y*-scale is multiplied by a fixed value, could yield results similar to those detailed here. However, the method presented allows one to maximize the expansion of the *y*-scale in a simple and reproducible manner. The method is not much different than scanning different ranges, where if one were to scan above or below the predominate ion, a partial spectrum similar to the ones described here would result. It should be recognized that this method is good for internal comparison, but would be of limited value in comparison to existing data found in mass spectra peak tables.

Gas chromatograph/mass spectrometry is a popular and efficient method to identify drugs of abuse. When the mass spectrum of a compound is not sufficiently informative to allow discrimination, other analytical techniques have been employed to obtain an identification. These other practices may be time-consuming, and in the case of infrared spectroscopy, difficult if the drug is contained in a matrix of other amines. The normalization procedure is a very quick and useful tool. With close observations of the normalized spectra, this procedure can aid in the identification of drugs with similar mass spectra.

References

1. Reyes RS. Identification of amphetamine and methamphetamine TMS derivatives via GC/MS. Microgram 1984;XVII(2):23–32.
2. Gan BK, Baugh D, Liu RH, Walia AS. Simultaneous analysis of amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) in urine samples by solid-phase extraction, derivatization, and gas chromatography/mass spectrometry. J Forensic Sci 1991;36(5):1331–41.
3. Dasgupta A, Gardner C. Distinguishing amphetamine and methamphetamine from other interfering sympathomimetic amines after various fluoro derivatization and analysis by gas chromatography-chemical ionization mass spectrometry. J Forensic Sci 1995;40(6):1077–81.
4. Bailey K, Legault D. The use of carbon-13 nuclear magnetic resonance spectra in the identification and authentication of monomethoxyamphetamines and dimethoxyamphetamines. J Forensic Sci 1981;26(1): 27–34.

Additional information and reprint requests:
Eric Buel, Ph.D.
Vermont Forensic Laboratory
P.O. Box 47
Waterbury, VT 05676