Technical Note

Analysis and Characterization of Psilocybin and Psilocin Using Liquid Chromatography - Electrospray Ionization Mass Spectrometry (LC-ESI-MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID)

Sandra E. Rodriguez-Cruz, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[email: sandra.e.rodriguez-cruz -at- usdoj.gov]

ABSTRACT: The rapid analysis of psilocybin and psilocin using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) is presented. Full-scan MS experiments provide molecular weight information, but little fragmentation. Similarly, collision-induced-dissociation (CID) experiments generate only a limited number of fragments. However, source-induced-dissociation (SID) experiments result in more extensive fragmentation. The combined results from these complementary techniques allows for the more complete characterization of psilocin and the thermally-labile psilocybin.

KEYWORDS: Psilocybin, Psilocin, Thermally-Labile, Liquid Chromatography-Mass Spectrometry (LC/MS), Tandem Mass Spectrometry, Collision-Induced-Dissociation, Source-Induced-Dissociation.

Introduction

Direct analysis of thermally-labile compounds using gas chromatography - mass spectrometry (GC/MS) is limited or impossible due to degradation caused by the high injector and column temperatures. Although derivatization is useful in many cases, direct analysis of the compounds of interest is always preferable. The development of the electrospray ionization (ESI) technique has enabled the transfer of thermally-labile compounds from solution into the gas phase without significant degradation [1]. The use of ESI in combination with liquid chromatography - mass spectrometry (LC/MS) techniques therefore provides a powerful analytical tool for the analysis of heat sensitive compounds.

A "classic" example of such a thermally labile compound is psilocybin, a powerful hallucinogen found in over 100 species of mushrooms, including *Psilocybe azurescens*, *Strophoria cubensis*, and *Psilocybe mexicana* [2-4]. Psilocybin is the phosphorylated ester of psilocin (Figure 1). The phosphate ester in psilocybin is delicate, and analysis of psilocybin-containing substrates by standard analytical techniques is therefore problematic. Because both psilocybin and psilocin are classified under Schedule I of the United States Controlled Substances Act, their analyses are important for forensic/law enforcement purposes.

Previous reports on the analysis of hallucinogenic mushrooms include descriptions of various extractions of the material, followed by instrumental analysis using liquid chromatography, gas chromatography, and mass spectrometry techniques [5-11]. In actuality, most of these analyses allow the detection of psilocin only, since the psilocybin did not survive the extraction and/or analysis. In addition, both psilocin and psilocybin have been indirectly analyzed following derivatization [12], and more recently, directly analyzed with the use of LC/MS

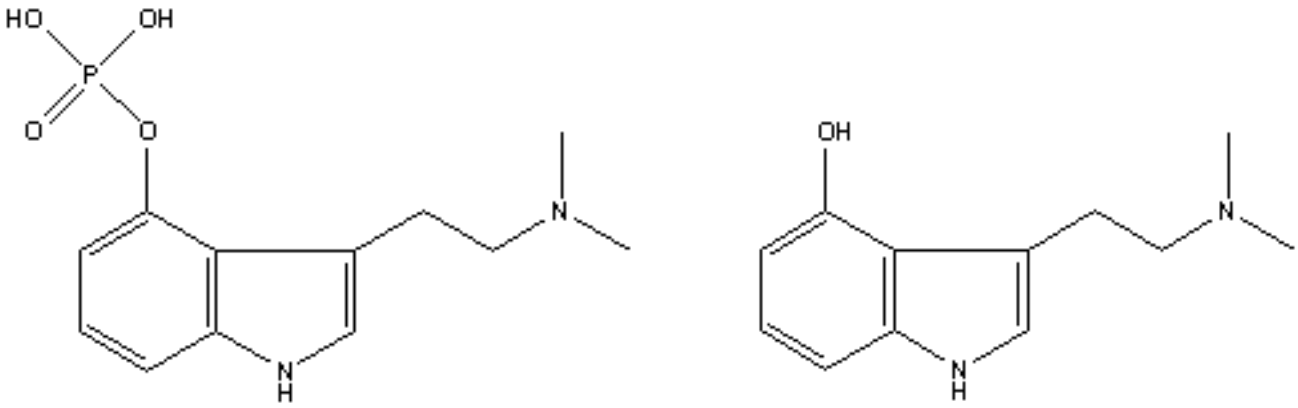

Figure 1. Chemical Structures of Psilocybin (Left) and Psilocin (Right).

and tandem mass spectrometry (MS/MS) techniques [13]. Unfortunately, even these advanced techniques give limited information beyond molecular weights.

However, when combined with multiple fragmentation techniques, LC-ESI-MS enables more complete characterization of thermally labile compounds. Collision-induced-dissociation (CID; MS/MS) can generate some fragment ions. For small compounds like tryptamines, a greater amount of dissociation and information can usually be obtained by performing source-induced-dissociation (SID) experiments, where ions are fragmented within the electrospray interface before they reach the mass analyzer.

Herein, a method is presented for the separation and characterization of psilocybin and psilocin using LC-ESI-MS in combination with CID (MS/MS) and SID experiments.

Experimental

Experiments were performed using a ThermoFinnigan LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an electrospray ionization source and interfaced to a Surveyor HPLC system (solvent pump, autosampler/column, and photodiode array detector).

Liquid chromatography conditions were investigated in order to provide for the best separation possible during the shortest analysis time. Separations were performed using a Phenomenex Prodigy column (150 x 4.6 mm; 5 μm), and an isocratic flow of 89 % Solvent A and 11 % Solvent B. Solvent A is H_2O with 0.1 % (v/v) formic acid, while Solvent B is acetonitrile with 0.1 % (v/v) formic acid. The eluent flow rate was 400 μL/minute. Standard solutions of psilocin (Sigma Chemical) and psilocybin (Alltech) were prepared at concentrations of 20 μg/mL in Solvent A.

Sample injections of 10 μL were loaded into the isocratic flow and introduced into the mass spectrometer using the ESI interface. The transfer capillary was maintained at a temperature of 250 °C, while the capillary and tube lens were kept at 20 and 15 V, respectively. Nitrogen (99 %; 100 ± 20 psi) was used as both the sheath and auxiliary gas, and operated at 50 and 20 units, respectively.

Mass spectrometry data were collected in the positive ion mode using the full-scan and tandem (MS/MS) modes in order to provide both molecular weight and structural information. MS/MS experiments were performed using

a standard collision energy of 35 eV. Source-induced-dissociation (SID) experiments were performed using variable energies between 25 and 40 eV. Helium (99.999 %; 40 ± 10 psi) was used as both the trapping and collision gas.

Instrument control, data collection and analysis were performed using the Xcalibur software (version 1.4) provided by the instrument manufacturer.

Results and Discussion

Figure 2 shows the total ion chromatogram (TIC), UV-based chromatogram, and full-scan ESI mass spectral data obtained during a 10 minute isocratic separation (11 % Solvent B). Psilocybin elutes at 5.5 minutes, while psilocin elutes at 7.4 minutes. Clear separation is obtained and the full-scan spectra show the pseudo-molecular $(M+H^+)^+$ ions for psilocybin and psilocin at *m/z* 285 and 205, respectively. The full-scan ESI spectrum for psilocybin also shows a peak at *m/z* 307, corresponding to the $(M+Na^+)^+$ ion. The ESI data for psilocin also shows a small fragment ion at *m/z* 160. This experiment allows for the separation, detection, and determination of the molecular weights for these two compounds.

Figure 3 shows the tandem (MS/MS) fragmentation data obtained during the chromatographic separation of psilocybin and psilocin. During standard collision-induced dissociation (CID) experiments at 35 eV, psilocybin dissociates into two main fragments. The fragment observed at *m/z* 205 corresponds to loss of a neutral phosphate moiety (HPO_3; 80 Da), while the fragment at *m/z* 240 results from the loss of neutral dimethylamine ($HN(CH_3)_2$; 45 Da). Dissociation of psilocin is dominated by the loss of dimethylamine, producing a fragment at *m/z* 160. The dissociation patterns observed for psilocybin and psilocin are typical of tryptamine-type fragmentations previously reported [14,15], and are also in agreement with recent tandem MS experiments using a triple-quadrupole mass analyzer [13].

Source-induced-dissociation (SID) experiments provide an alternative fragmentation technique for compounds that show a limited number of fragments under MS/MS conditions. During SID, electrospray-generated ions are subjected to high-energy collisions with the background gas within the relatively high-pressure capillary-skimmer region of the ionization interface. As a result, characteristic fragments are generated and mass analysis provides additional structural information. Figures 4 and 5 show SID data obtained for psilocybin and psilocin, respectively, using dissociation energies of 25, 30, 35, and 40 eV.

For psilocybin, SID experiments result in the formation of multiple fragments. In addition to the fragments at *m/z* 240 and 205 observed with CID, other characteristic fragments are observed at *m/z* 222, 160, 142, and 115. The former three fragments correspond to loss of H_2O, HPO_3, and H_3PO_4 from the *m/z* 240 species, while the peak at *m/z* 115 is characteristic of the indole moiety. The fragment at *m/z* 160 can also be generated from the loss of $HN(CH_3)_2$ from *m/z* 205. As observed in the SID spectra, the sodiated psilocybin ion at *m/z* 307 does not undergo significant dissociation under these conditions. This is probably reflective of the greater stability of this species, due to the higher affinity of the phosphate group for sodium.

Increased fragmentation is also observed from SID experiments on psilocin. After production of *m/z* 160, subsequent dissociation reactions result in the appearance of fragments at *m/z* 142 and 132, due to loss of H_2O and $CH_2{=}CH_2$, respectively. The fragments observed at *m/z* 115 and 117 are again characteristics of the indole group, with and without the loss of H_2.

Conclusions

The presented LC-ESI-MS techniques allow for the direct, facile separation and identification of psilocybin and psilocin. The LC conditions used separated the two compounds in less than 8 minutes. Mass spectrometry

experiments in the full-scan and MS/MS mode provided molecular weight and partial structural information. Source-induced-dissociation experiments provided complementary fragment information, allowing for a much more complete structural characterization of the two species. The presented techniques illustrate the utility of LC-ESI-MS, CID, and SID experiments for the analysis and characterization of thermally-labile compounds.

References

1. Cole RB. Electrospray ionization mass spectrometry: Fundamentals, instrumentation and applications. John Wiley and Sons, New York, 1997.

2. Drug identification bible. Amera-Chem, Inc., Grand Junction, Colorado, 2002.

3. Gahlinger P. Illegal drugs: A complete guide to their history, chemistry, use, and abuse. Plume, New York, 2004.

4. Perrine DM. The chemistry of mind-altering drugs: History, pharmacology and cultural context. American Chemical Society, Washington, D.C., 1996.

5. Thomson BM. Analysis of psilocybin and psilocin in mushroom extracts by reverse phase HPLC. Journal of Forensic Sciences 1980;25:779.

6. Casale J. An aqueous-organic extraction method for the isolation and identification of psilocin from hallucinogenic mushrooms. Journal of Forensic Sciences 1985;30:247.

7. Lee RE. Techniques for the rapid isolation and identification of psilocin from psilocybin containing mushrooms. Journal of Forensic Sciences 1985;30:931.

8. Phelan CP. Identification of psilocin and bufotenine via GC/IRD. Microgram 1999;32:83 (Note: Law Enforcement Restricted).

9. Gross TS. Detecting psychoactive drugs in the developmental stages of mushrooms. Journal of Forensic Sciences 2000;45:527.

10. Gross TS. Psychotropic drugs in developmental mushrooms: A case study review. Journal of Forensic Sciences 2002;47:1298.

11. Saito K, Toyo'oka T, Fukushima T, Kato M, Shirota O, Goda Y. Determination of psilocin in magic mushrooms and rat plasma by liquid chromatography with fluorimetry and electrospray ionization mass spectrometry. Analytica Chimica Acta 2004;527:149.

12. Timmons JE. The identification of psilocin and psilocybin using gas chromatography - mass spectrometry. Microgram 1984;17:28 (Note: Law Enforcement Restricted).

13. Kamata T, Nishikawa M, Katagi M, Tsuchihashi H. Liquid chromatography - mass spectrometric and liquid chromatography - tandem mass spectrometric determination of hallucinogenic indoles psilocin and psilocybin in "magic mushrooms" samples. Journal of Forensic Sciences 2005;50:336.

14. Roesner P. Mass spectra of designer drugs. John Wiley and Sons, Inc., New York, 2003.

15. Rodriguez-Cruz SE. Analysis and characterization of designer tryptamines using electrospray ionization mass spectrometry (ESI-MS). Microgram Journal, this issue, page 107.

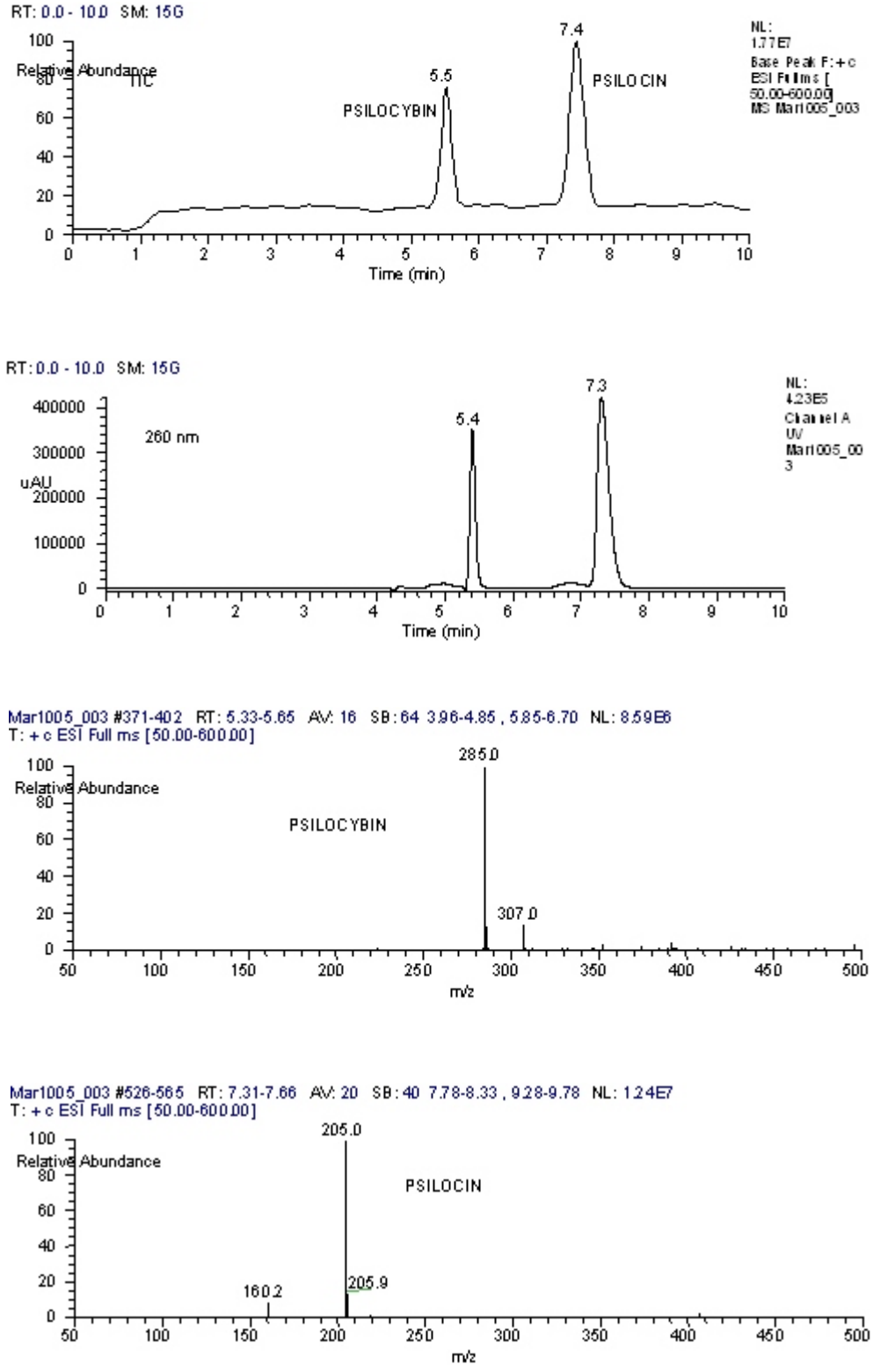

Figure 2. Total Ion Chromatogram, UV Chromatogram, and Full-Scan ESI Spectra Showing the Separation and Detection of Psilocybin (MW = 284) and Psilocin (MW = 204).

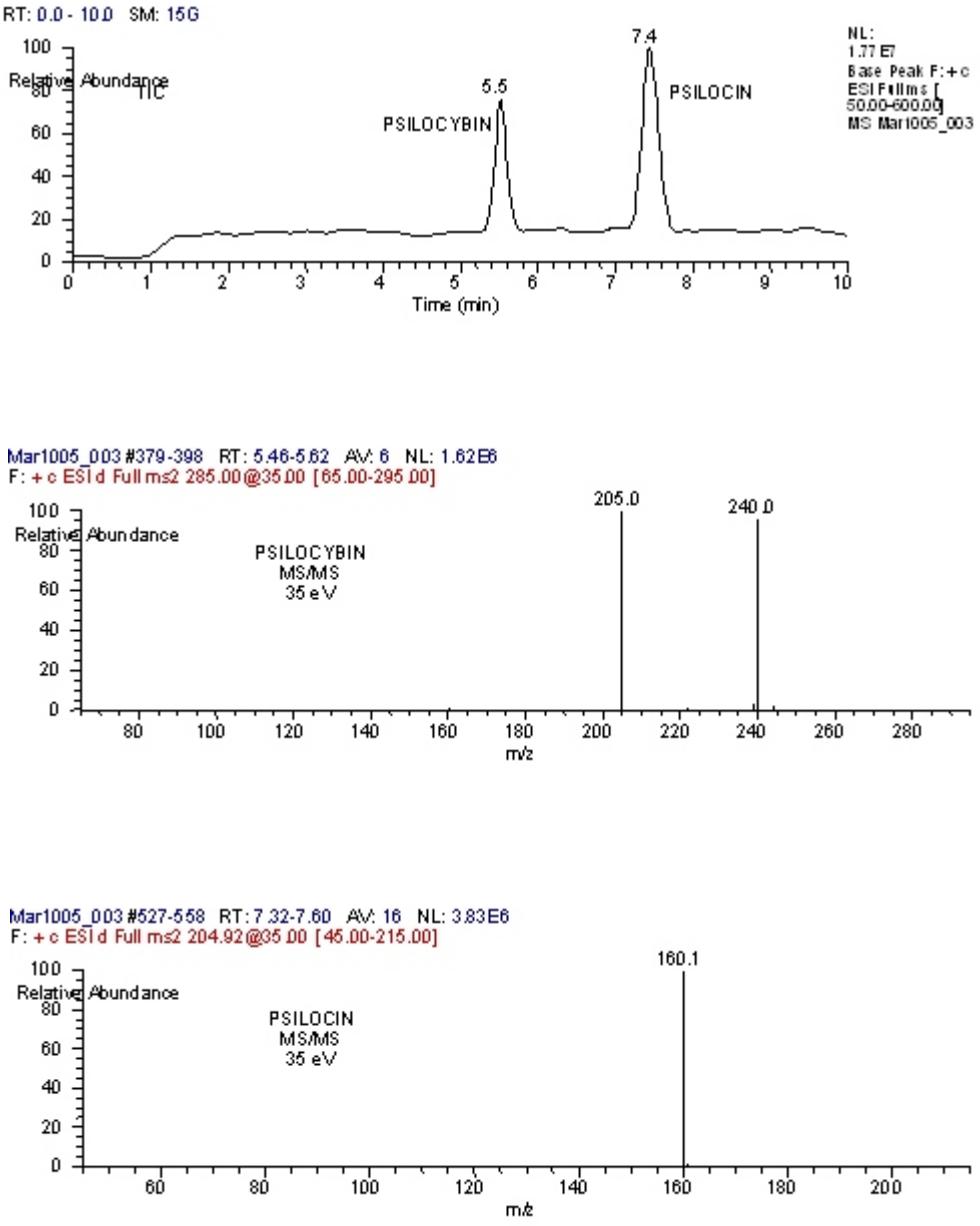

Figure 3. Total Ion Chromatogram and MS/MS Spectra Showing the Fragmentation of Psilocybin and Psilocin under Standard Collision-Induced-Dissociation Conditions at 35 eV.

* * * * *

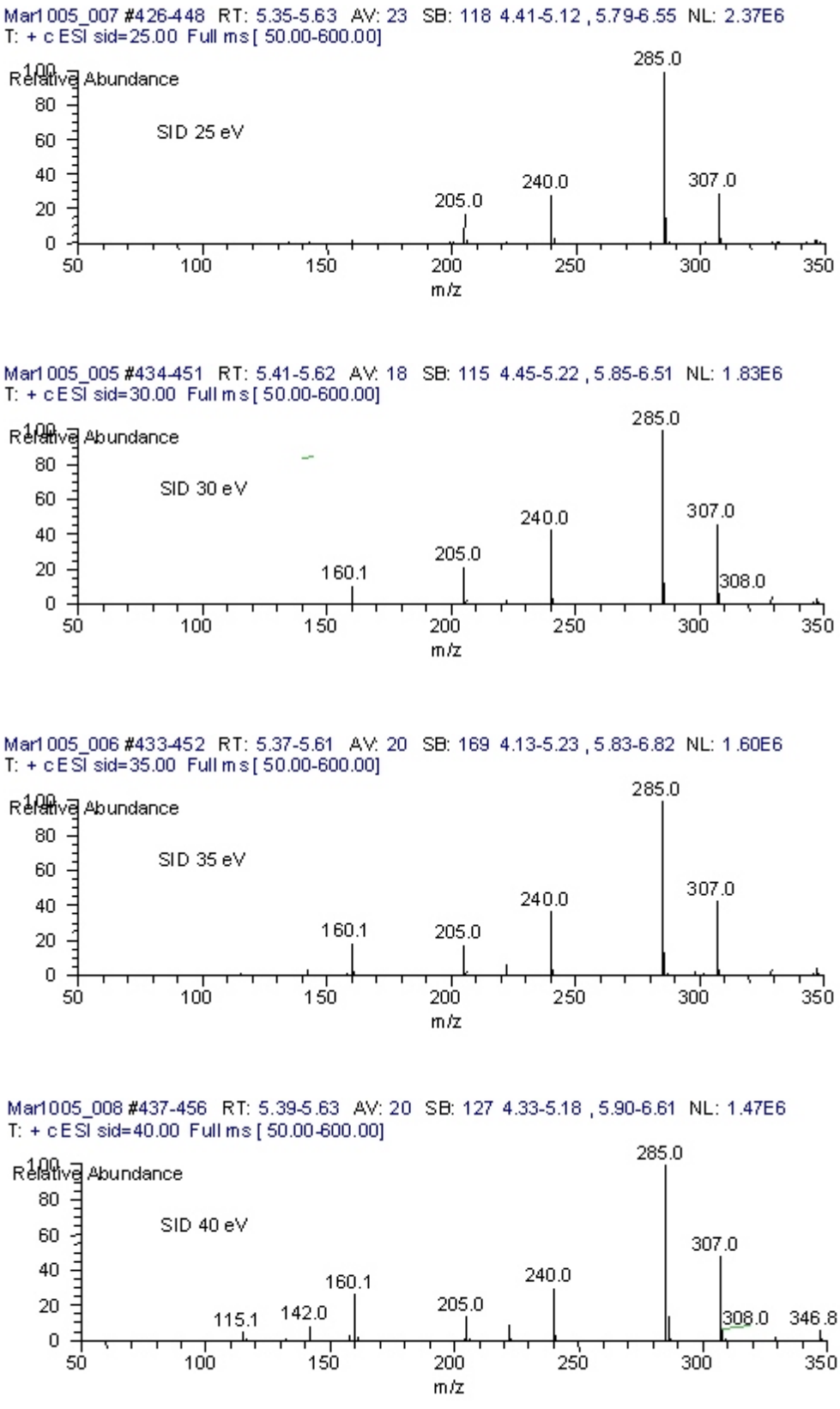

Figure 4. SID Spectra for Psilocybin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.

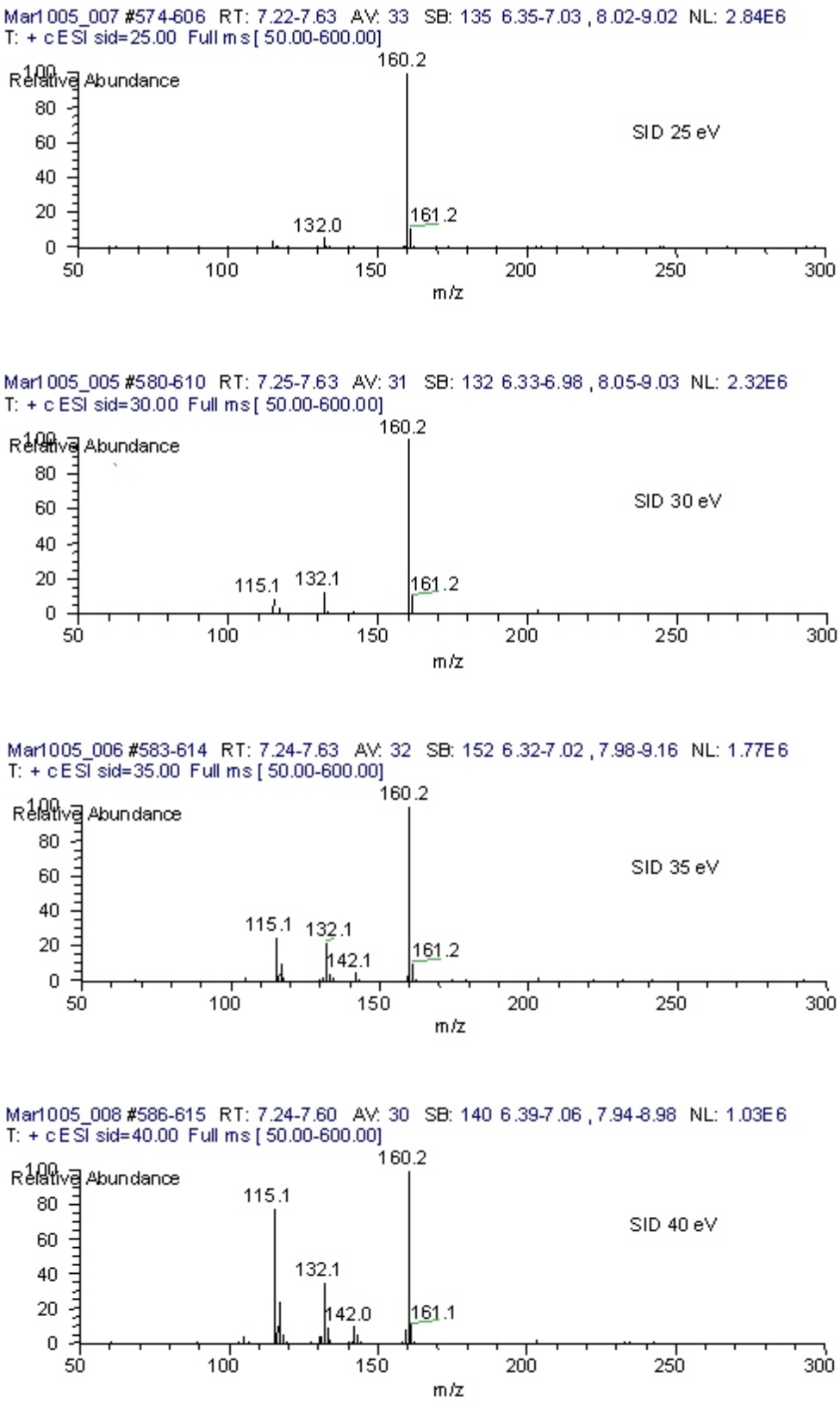

Figure 5. SID Spectra for Psilocin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.