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Ion Spray LC/MS Determination of Perfluoro Analytical Standards Provided by 3M Medical Department

Prepared for 3M Medical Department, Toxicology Services,
St. Paul, MN 55133-3220

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Advanced BioAnalytical Services, Inc.

Date: August 30, 1995

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SIGNATURE PAGE

Title: Ion Spray LC/MS Determination of Perfluoro Analytical Standards Provided by 3M Medical Department

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SUMMARY

Five perfluoro compounds including T-6293, T-6294, T-6295, fose amide and perfluorooctanamide were analyzed by full-scan ion spray LC/MS for composition identification and ambient temperature stability in DMSO and water (only for T-6293). The on-line LC/MS determination of the listed test articles was carried out under unit mass resolution conditions following mass axis calibration using PPG reference compounds. Chromatography was performed on a 2 x 100 mm Deltabond™ C8 column using linear gradient elution with acetonitrile-water from 5:95 to 95:5 in 15 minutes. The mass spectrometer was operated in the negative ion mode. The LC/MS experiments showed that all five perfluoro compounds contain some impurities. In addition, all five perfluoro compounds are stable in DMSO and water for at least a week at ambient temperature at a concentration of 10 mM. No apparent degradation was observed by LC/MS analyses of these samples. Attempts to obtain molecular ion information for compounds T-6292 and butyl fose by ion spray and atmospheric pressure chemical ionization (APCI) were unsuccessful. However, poor response from both ionization modes did not provide sufficient sensitivity for the LC/MS analysis of these two compounds.

1. INTRODUCTION

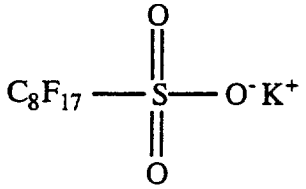
Seven perfluoro compounds including T-6292, T-6293, T-6294, T-6295, fose amide, butyl fose and perfluorooctanamide were the target molecules for qualitative composition and stability determination. Among them, five perfluoro compounds including T-6293, T-6294, T-6295, fose amide and perfluorooctanamide were analyzed by full-scan ion spray LC/MS for composition identification and ambient temperature stability in DMSO and water (only for T-6293). The on-line LC/MS determination of the listed test articles was carried out under unit mass resolution conditions following mass axis calibration using PPG reference compounds. Chromatography was performed on a 2 x 100 mm Deltabond™ C8 column using linear gradient elution with acetonitrile-water from 5:95 to 95:5 in 15 minutes. The mass spectrometer was operated in the negative ion mode. The LC/MS experiments showed that all five perfluoro compounds contain some impurities. In addition, all five perfluoro compounds are stable in DMSO and water for at least a week at ambient temperature at a concentration of 10 mM. No apparent degradation was observed by LC/MS analyses of these samples. Attempts to obtain molecular ion information for compounds T-6292 and butyl fose by ion spray and atmospheric pressure chemical ionization (APCI) were unsuccessful. Poor response from both positive and negative ionization modes did not provide sufficient sensitivity for the LC/MS analysis of these two compounds.

1.1 Study Objectives

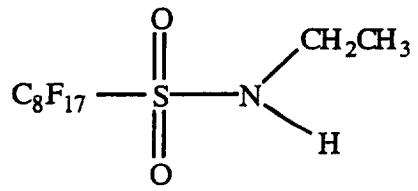
The objectives of this study were:

* to qualitatively determine the composition of each perfluoro compound.
* to qualitatively determine the stability of each compound in DMSO or water (for T-6293 only) as a stock solution at 10 mM concentration.

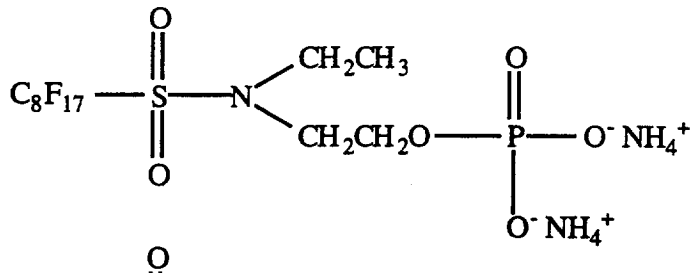
1.2 Chemical Structures, Names or 3M Identifications, and Molecular Weights



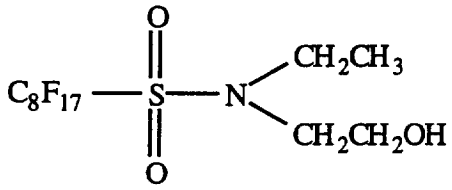
T-6295
MW = 538.22
anion m/z = 499



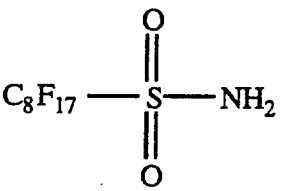
T-6294
MW = 527.20
anion m/z = 526



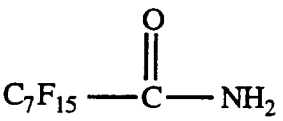
T-6293
MW = 685.29
monoanion m/z = 650



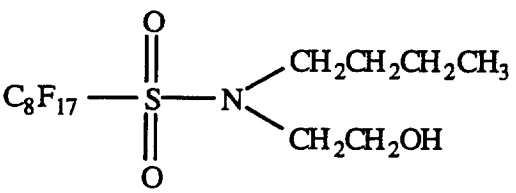
T-6292
MW = 571.25



fose amide
MW = 499.14
anion m/z = 498



Perfluorooctanamide
MW = 413.09
anion m/z = 412



butyl fose
MW = 599.31

2. MATERIALS AND METHODS

2.1 Chemicals and Materials

All seven perfluoro compounds were provided by 3M Medical Department, Toxicology Services, St. Paul, MN 55133-3220. Methyl sulfoxide (DMSO, Cat #27,043-1) was obtained from Sigma-Aldrich Chemicals, Milwaukee, WI 53233. Acetonitrile (Cat #015-4) was obtained from

Baxter/Scientific Products, McGaw Park, IL 60085. Ammonium Acetate (Cat #JT0596-01) was purchased from J.T. Baker, Danvers, MA 01923. High purity (NANOpure) water was made by Barnstead Model D7331 Ultrapure Water System, Dubuque, IA 52001.

All materials and equipment may be substituted by equivalent materials and equipment.

Fisher Vortex Genie 2™, Cat #12-812, Fisher Scientific, Springfield, NJ 07081

Microbalance, Model MT5, Mettler-Toledo, Inc., Hightstown, NJ 08520

Siliconized Graduated Tubes, 2 mL, Cat #M-720, Phenix Research Products, Hayward, CA 94545

Pipettes, Cat #P-1000, P-200, P-100, P-20, Rainin Instrument Co., Woburn, MA 01888

Siliconized Green Slick Tips, 1 mL, Cat #TGS-415BRS, Phenix Research Products, Hayward, CA 94545

Siliconized Clear Tips, 250 µL, Cat #T-051 BRCS, Phenix Research Products, Hayward, CA 94545

Polypropylene autosampler vials (siliconized in house), Cat #500 102, Sun Brokers, Inc., Wilmington, NC 28402

Snap Cap, 11 mm, Cat #69158-P, Scientific Resources Inc., Eatontown, NJ 07724

Solvent Filtration Apparatus, Cat #32-00610, Krackeler Scientific, Albany, NY 12201

Vacuum Pump, Cat #54-907, VWR Scientific, Rochester, NY 14603

0.45 µm Nylon Titan Membrane Filters, Cat #74547-NN, Scientific Resources Inc., Eatontown, NJ 07724

Liquid Nitrogen, Cat #SS180N-NIT4052, Empire Airgas, Elmira, NY 14901

U.H.P. Nitrogen, Empire Airgas, Elmira, NY 14901

U.H.P. Argon, Empire Airgas, Elmira, NY 14901

Fused-silica, 100 µ i.d. x 170 µ o.d., Polymicro Technologies, Phoenix, AZ 85023

PEEK tubing, 0.007" i.d. x 1/16" o.d., Cat. #1536, Upchurch Scientific, Inc., Oak Harbor, WA 98277

2.2 LC/MS Instrumentation

The liquid chromatography part of the system included a Hewlett-Packard Model 1090L pump, Hewlett-Packard, Avondale, PA 19311. Deltabond™ C8 columns, 5 µm, 2 x 100 mm, and Deltabond™ C8 guard cartridges (5 µm, 2 x 10 mm) were obtained from Keystone Scientific, Inc.,

Bellefonte, PA 16823. The mass spectrometer was a PE-SCIEX API III$^+$ from PE-SCIEX, Thornhill, Ontario.

HPLC Conditions:

Flow Rate		200 µL/min	
Column Temperature		Ambient	
Injection Volume		1 µL	
Column		2 x 100 mm Deltabond™ C8	
Guard Cartridge		2 x 10 mm Deltabond™ C8	
Pressure		70 bar	
Eluents	t (min)	%acetonitrile	%water
	0	5	95
	15	95	5

This program was used for the analyses of T-6294, T-6295, fose amide and perfluorooctanamide. For the analysis of T-6293 samples, water was replaced by 10 mM ammonium acetate with the same gradient program.

Mass Spectrometer Conditions:

Ion Spray Voltage	-4200 V
Orifice Voltage	-90 V (-70 V for T-6293)
Declustering Potential	-60 V (-40 V for T-6293)
Multiplier Voltage	4000 V
Curtain Gas (U.H.P. N_2)	1.4 L/min
Nebulizer Gas (N_2)	50-70 psi
Interface Heater	60 °C
Turbospray Temperature	500 °C
Turbospray Gas Flow	4.0 L/min
Dwell Time	1 msec
Mass Scan Range	100-900 Da
Scan Rate	1.69 sec/scan

Count Controller (CC) 10

Mass calibration of Q1 in the range of 100-900 was performed daily by the infusion of a 10^{-4} M PPG (polypropylene glycols, average MW 425, 1000 and 2000) calibration solution (PE–SCIEX API III$^+$ operator's manual) at a flow of 10 µL/min. Calibration was performed on m/z 59.0, 325.3, 500.38 and 906.7. The mass spectrometric parameters, MS/MS peak widths and sensitivity are optimized daily. Peak widths are approximately 0.7 amu at half-height in the single MS mode.

2.3 Preparation of Stock and Test Solutions

Stock Solutions: Accurately weigh approximately 5 mg of one of the perfluoro compounds (assuming purity 100%) on a micro balance in a siliconized graduated tube (2 mL). Dissolve the solid sample in an appropriate amount of DMSO or water (for T-6293 only) to yield a 10 mM solution. Store the solution at ambient temperature.

Test Solutions: Carefully take 10 µL of the 10 mM stock solution from the top, middle and bottom parts of the solution, and dilute them with DMSO or water (for T-6293 only) by 10- to 100-fold to give test solutions with concentrations of 100-1000 µM. Vortex-mix the diluted solutions, and analyze them immediately after preparation.

The test solutions with same concentrations for the analytes were prepared on day zero and day seven in the same way from the same stock solution. The stock solutions were stored at ambient temperature on the lab bench without disturbance.

3. RESULTS AND DISCUSSION

As requested by 3M, the polypropylene tubes and vials used in this work were siliconized. The pipette tips used in this work were also siliconized. The LC/MS conditions for the analyses of different parts of a solution on both day zero and day seven were the same. To avoid cross contamination, solvent blanks (DMSO or water) were injected before the injection of a different type of test solution. No LC/MS data were obtained for compounds T-6292 and butyl fose, because the molecular ion signal was too weak to be used to perform LC/MS experiments.

The on-line LC/MS determination of the five compounds tested was carried out under unit mass

resolution conditions following mass axis calibration using PPG reference compounds. To achieve sufficiently fast full-scan acquisition scan rates for these relatively higher molecular weight compounds, a compromise of 0.5 Da step size acquisition was used in these experiments. These conditions produce plots of corresponding mass spectra from the proprietary PE SCIEX software that do not include the isotopic peaks. Mass spectral plots of individual test article mass spectra from these acquisitions show reliable molecular weight measurements. In particular, differentiation of molecular weights differing by only one dalton, e.g. the sulfonamide vs. the sulfonic acid at m/z 498 and 499, may be reliably determined under these conditions.

3.1 T-6295

A typical total ion chromatogram (TIC) from the injection of 1 µL of DMSO is shown in Figure 1. The TIC from the injections of top, middle and bottom parts of the T-6295 solution on day zero and day seven were summarized in Figures 2-3. Extracted ion chromatograms from the injection of 1 µL of day zero test solution (100 µM) diluted from the top part of the stock solution are shown in Figure 4. Full-scan single MS spectra of the components detected from Figure 4 are shown in Figures 5-11. The identical mass spectra in Figure 9 for peaks 5a and 5b suggest that these two peaks are likely from two structural isomers. By carefully examining the TIC from the injections of solvent blank and different parts of the T-6295 solutions on both day zero and day seven, there is no apparent difference among the three parts of the solution and there is no apparent change in the composition of the stock solution at ambient temperature for a week. Although trace impurities at m/z 299, 349, 399, 449, 549 and 607 are detected in T-6295, the major species present is the m/z 499 anion.

3.2 T-6294

The TIC from the injections of top, middle and bottom parts of the T-6294 solution on day zero and day seven were summarized in Figures 12-13. Extracted ion chromatograms from the injection of 1 µL of day seven test solution (100 µM) diluted from the top part of the stock solution are presented in Figure 14. Full-scan single MS spectra of the components detected from Figure 14 are shown in Figures 15-19. By carefully examining the TIC from the injections of solvent blank and different parts of the T-6294 solutions, it appears that the bottom part of the solution contains fewer impurities. In addition, ions at m/z 297.5, 311.5, 325.5 and 339.5 showed higher relative responses (compared to the ion at m/z 526 for $[M-H]^-$ of T-6294) for the day seven

solutions than those obtained for the day zero solutions (See Figures 12 and 13 in the regions of 5.6-8.5 min.). Again, the m/z 526 anion is the major species in the sample.

3.3 T-6293

T-6293 was not soluble in DMSO. Therefore, water was used to prepare the stock and test solutions for T-6293. A typical TIC from the injection of 1 μL of water is shown in Figure 20. Total ion chromatograms from the injections of top, middle and bottom parts of the T-6293 solution on day zero and day seven are summarized in Figures 21-22. Extracted ion chromatograms from the injection of 1 μL of day seven test solution (1 mM) diluted from the bottom part of the stock solution are presented in Figure 23. Full-scan single MS spectra of the components detected from Figure 23 are shown in Figures 24-26. Components at m/z 650, 630 and 369 were detected. Based on infusion experiments, the ion at m/z 526 in Figure 25 is a fragment of m/z 650. By carefully examining the TIC from the injections of solvent blank and different parts of the T-6293 solutions on both day zero and day seven, there is no apparent difference among the three parts of the solution and there is no apparent change in the composition of the stock solution at ambient temperature for a week, although the chemical background for the day seven solutions seems to be noisier possibly due to lower sensitivity of the mass spectrometer. For unknown reasons, the chemical background for the day zero top and middle solutions are somewhat higher than that for the day zero bottom solution (see Figure 21).

3.4 Fose Amide

The TIC from the injections of top, middle and bottom parts of the solution of fose amide on day zero and day seven were summaried in Figures 27-28. Extracted ion chromatograms from the injection of 1 μL of day zero test solution (100 μM) diluted from the top part of the stock solution are presented in Figure 29. Full-scan single MS spectra of the components detected in Figure 29 are shown in Figures 30-36. Components at m/z 606, 499, 498, 398, 339.5, 325.5, and 311.5 were detected. Peaks marked with an X are from the chemical background of the LC eluent (see Figures 34 and 36). Interestingly, the top portion of the day zero solution showed much higher relative response for the ions at m/z 499, 339.5, 325.5 and 311.5 than those obtained from the middle and bottom portions of the same day solution. On the contrary, the day seven solution showed the opposite trend for the responses of those ions. The ion at m/z 499 appeared at two different retention times (see peak 1a and 1b in Figure 29). Peak 1a is possibly from an impurity, while peak 1b is the isotopic form of the major component at m/z 498 (see Figures 30 and 35). By

carefully examining the TIC from the injections of solvent blank and different parts of the solutions of fose amide on both day zero and day seven, there is no significant difference between the three parts of the solution and there is no apparent change in the composition of the stock solution at ambient temperature for a week.

3.5 Perfluorooctanamide

The TIC from the injections of top, middle and bottom parts of the solution of perfluorooctanamide on day zero and day seven are summarized in Figures 37-38. Extracted ion chromatograms from the injection of 1 μL of day zero test solution (1 mM) diluted from the top part of the stock solution are presented in Figure 39. Full-scan single MS spectra of the components detected in Figure 39 are shown in Figures 40-46. Major components at m/z 412, 392, 369, 339.5, 325.5, 311.5 and 297 were detected. Peaks marked with an X are from the chemical background of the LC eluent (see Figure 46). Again, the top portion of the day zero solution showed much higher relative response for the ions at m/z 339.5, 325.5 and 311.5 than those obtained from the middle and bottom portions of the same day solution. The relative responses for the ions at m/z 339.5, 325.5 and 311.5 for the day seven solution were significantly lower than those obtained for the day zero solution. It is clear from Figure 39 that this sample has several components present in addition to the test article of interest.

4. CONCLUSION

Five perfluoro compounds were analyzed by ion spray LC/MS. In general, all five compounds are stable in DMSO or water (for T-6293 only). No significant degradation products were detected for any of the compounds analyzed. However, it appears that the low molecular weight impurities are not uniformly distributed in some solutions.

5. FIGURES

Figure 1. Total Ion Chromatogram (TIC) for the Injection of 1 μL of DMSO

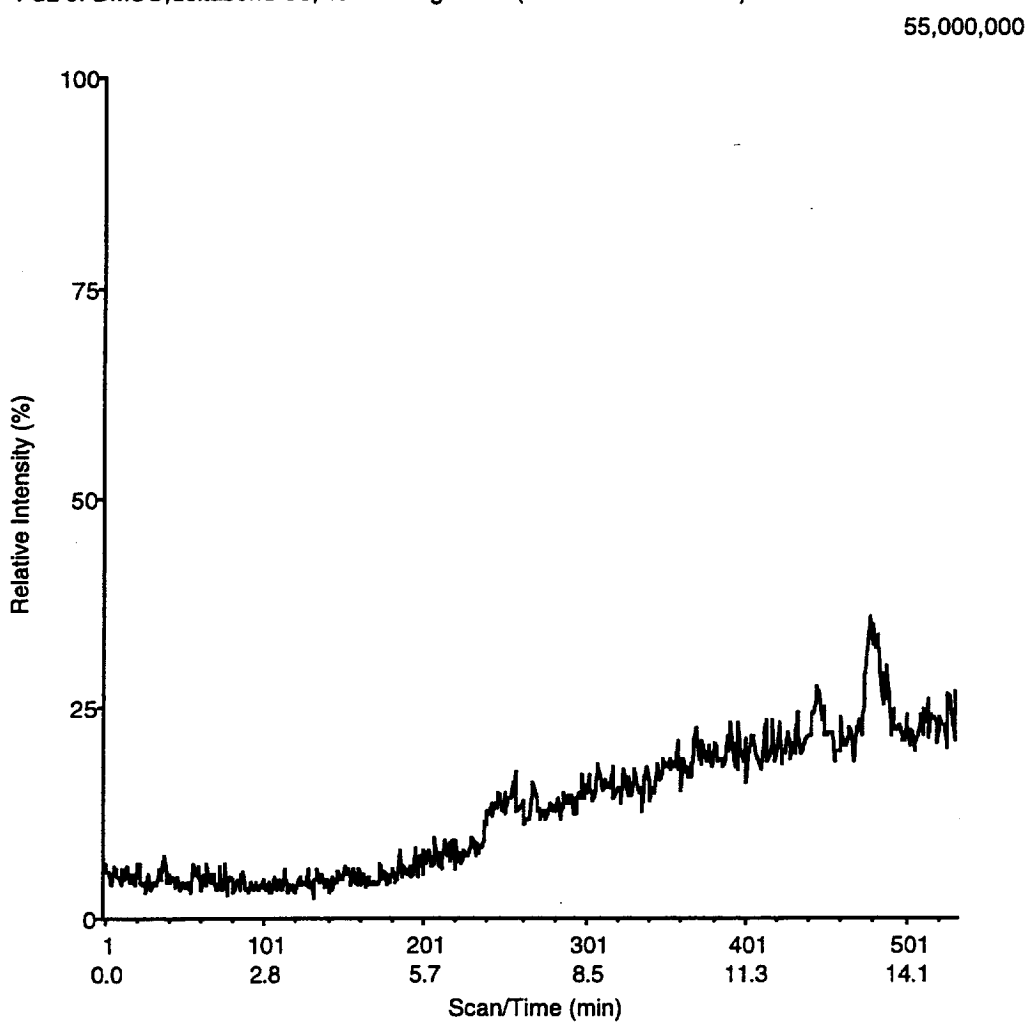


Figure 2. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Zero Solution of T-6295

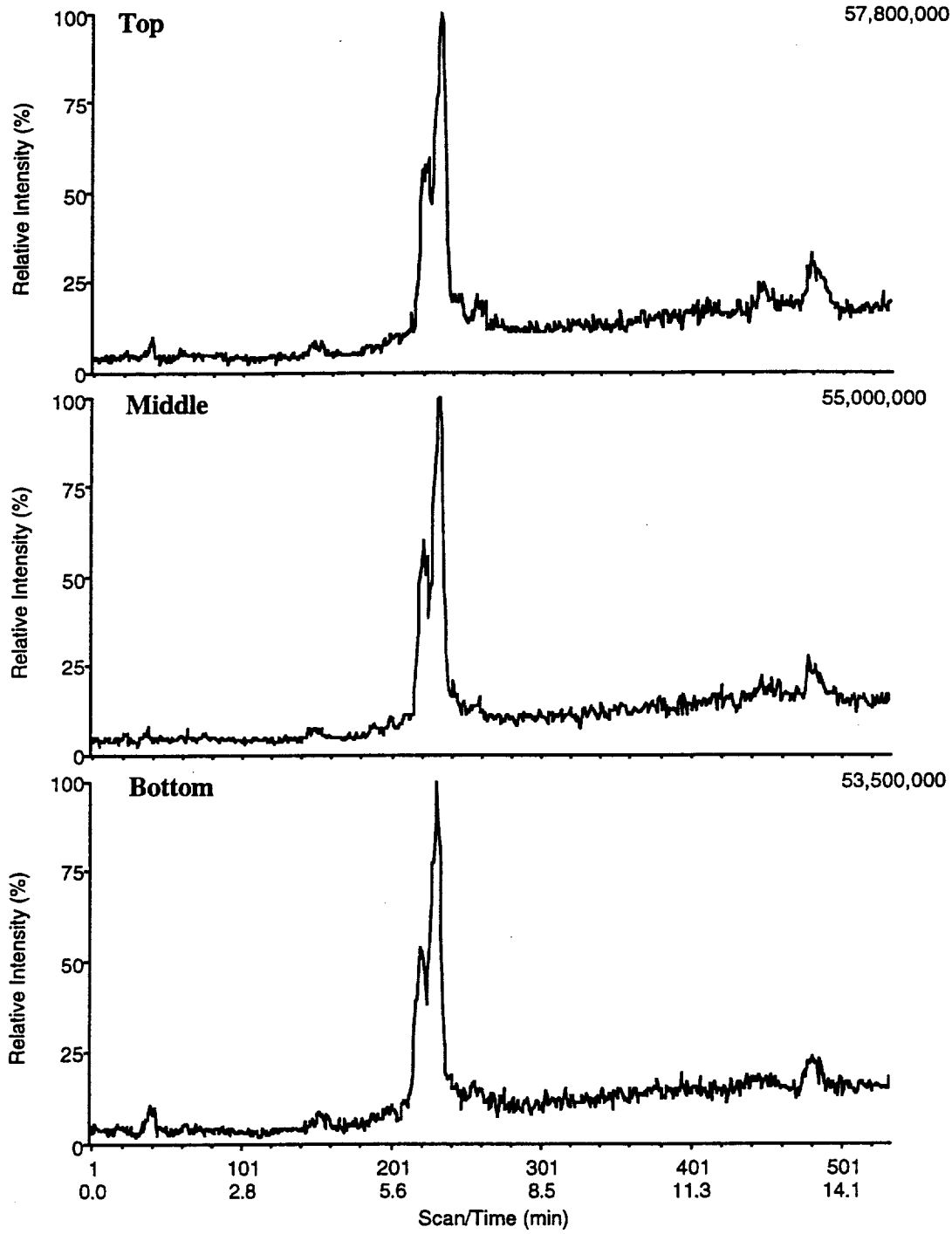


Figure 3. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Seven Solution of T-6295

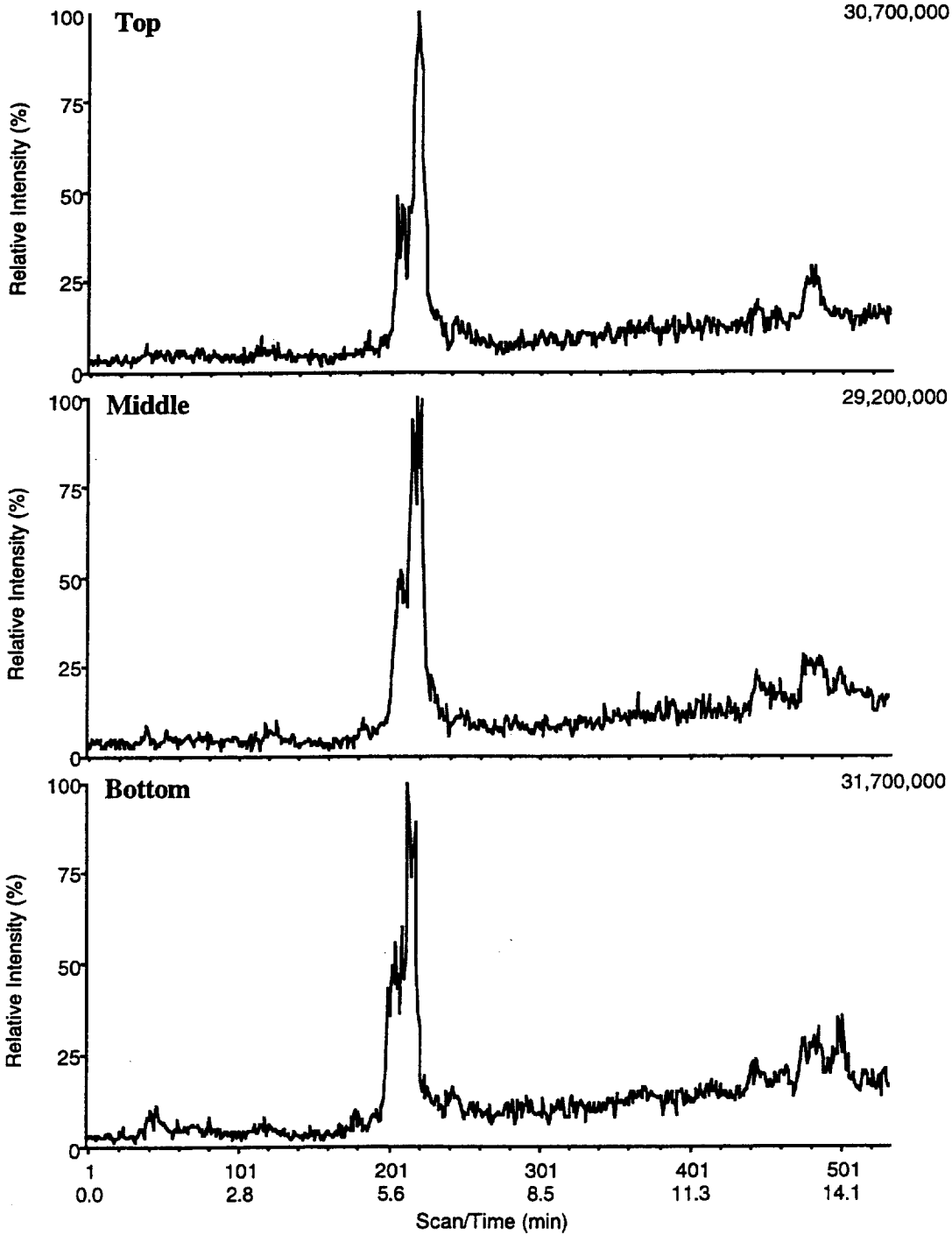


Figure 4. Extracted Ion Chromatograms for the Injection of 1 μL of Day Zero Top Solution of T-6295

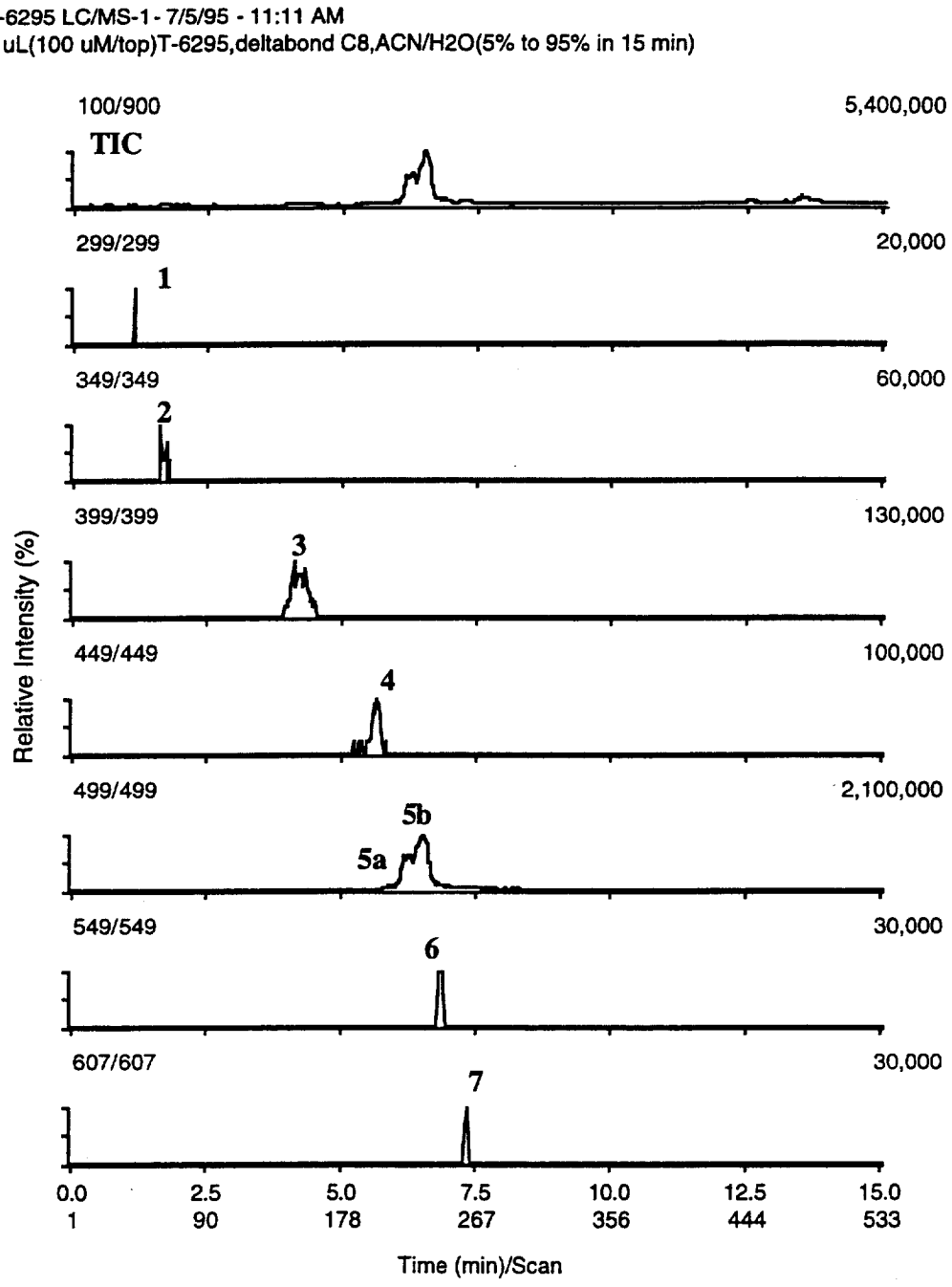


Figure 5. Full-Scan Mass Spectrum of Peak 1 in Figure 4

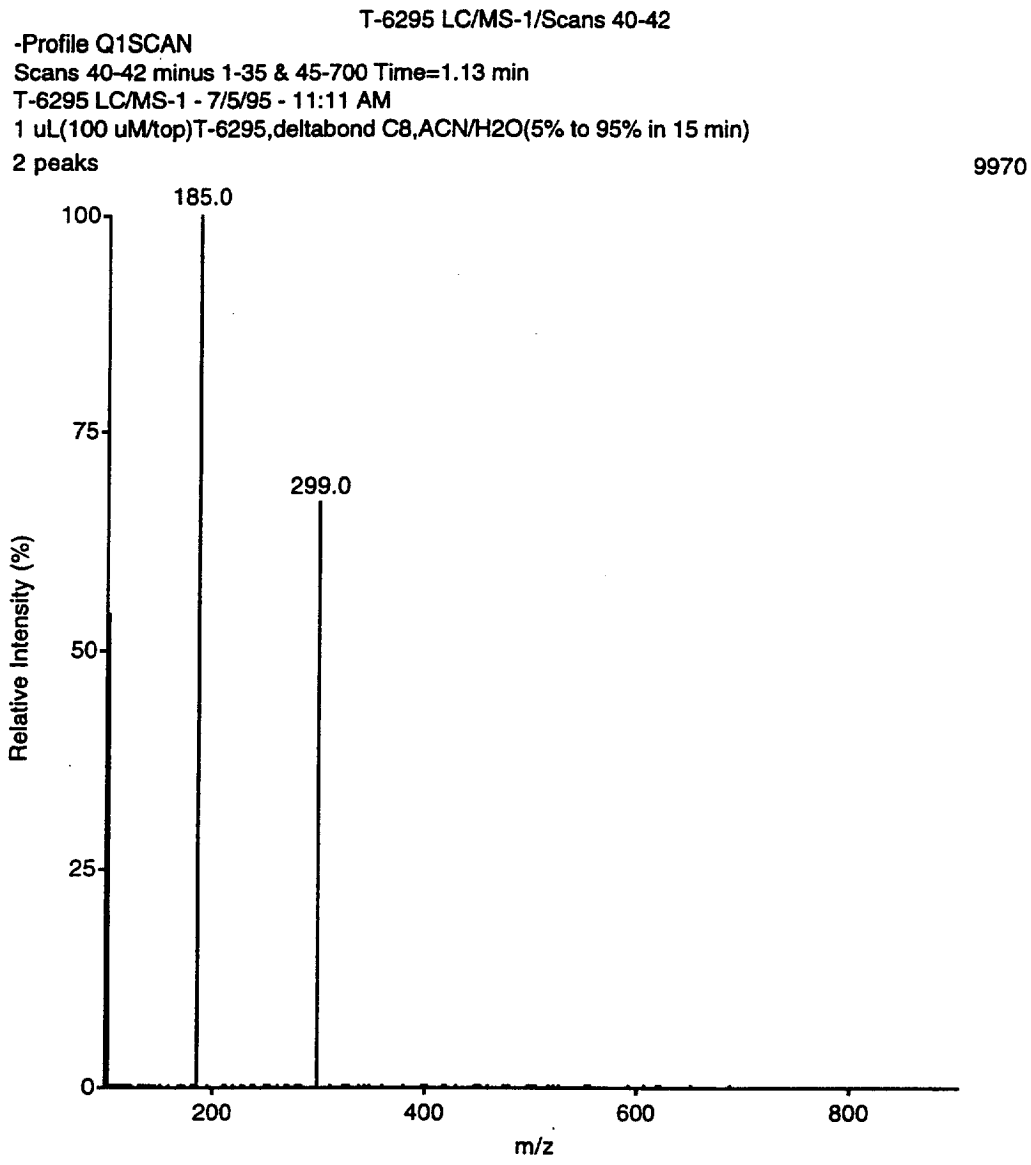


Figure 6. Full-Scan Mass Spectrum of Peak 2 in Figure 4

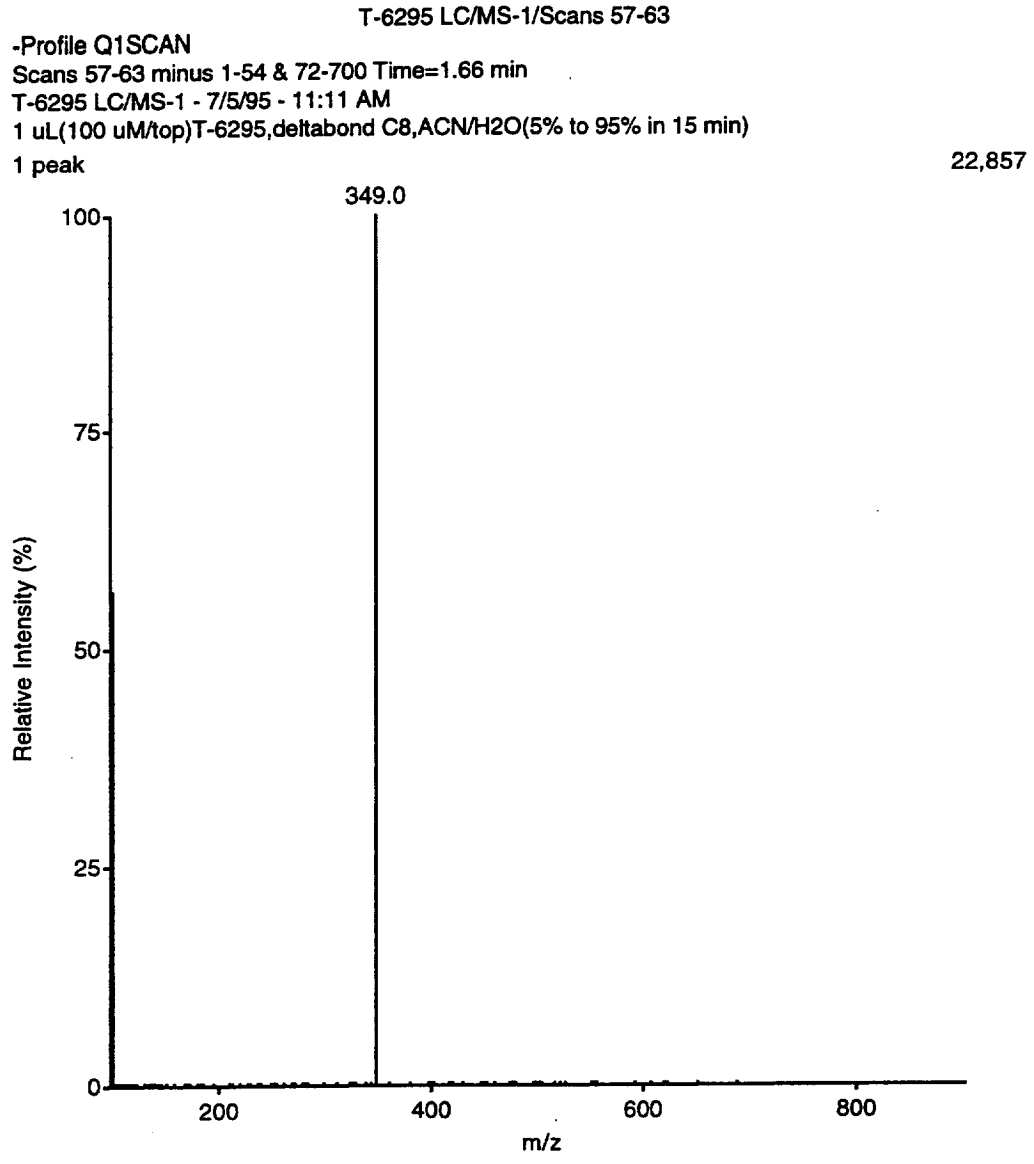


Figure 7. Full-Scan Mass Spectrum of Peak 3 in Figure 4

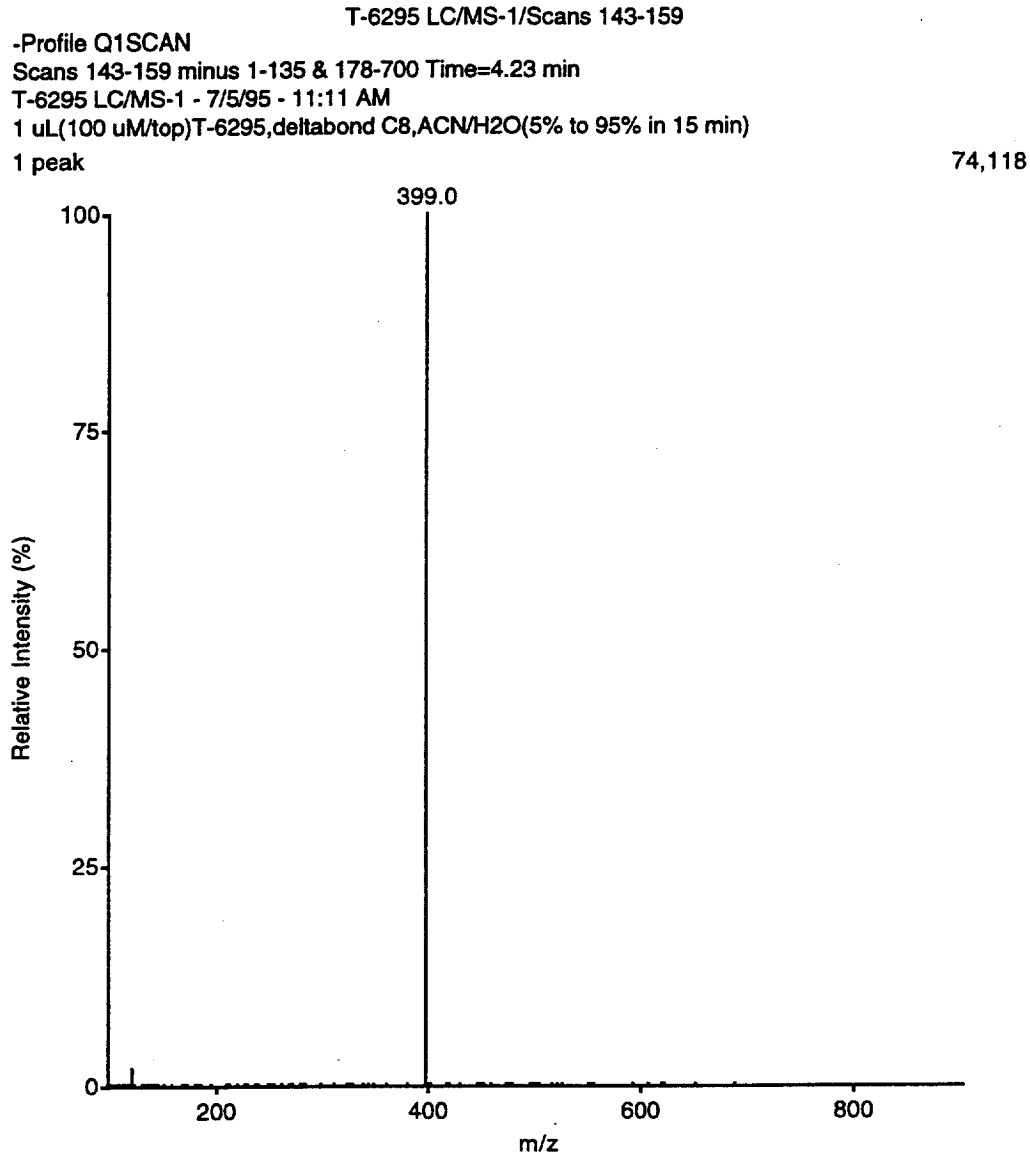


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Figure 8. Full-Scan Mass Spectrum of Peak 4 in Figure 4

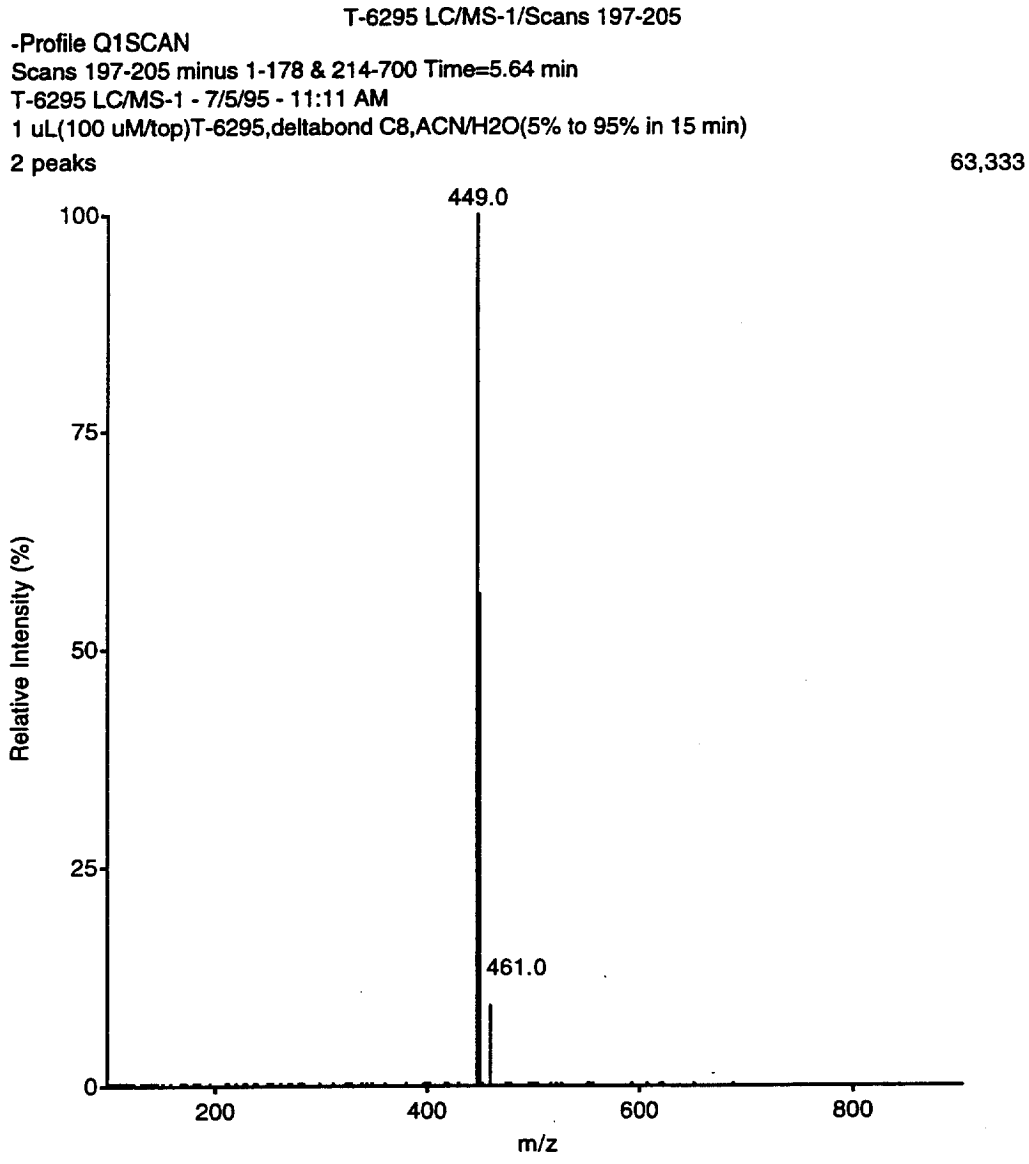

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Figure 9. Full-Scan Mass Spectra of Peaks 5a and 5b in Figure 4

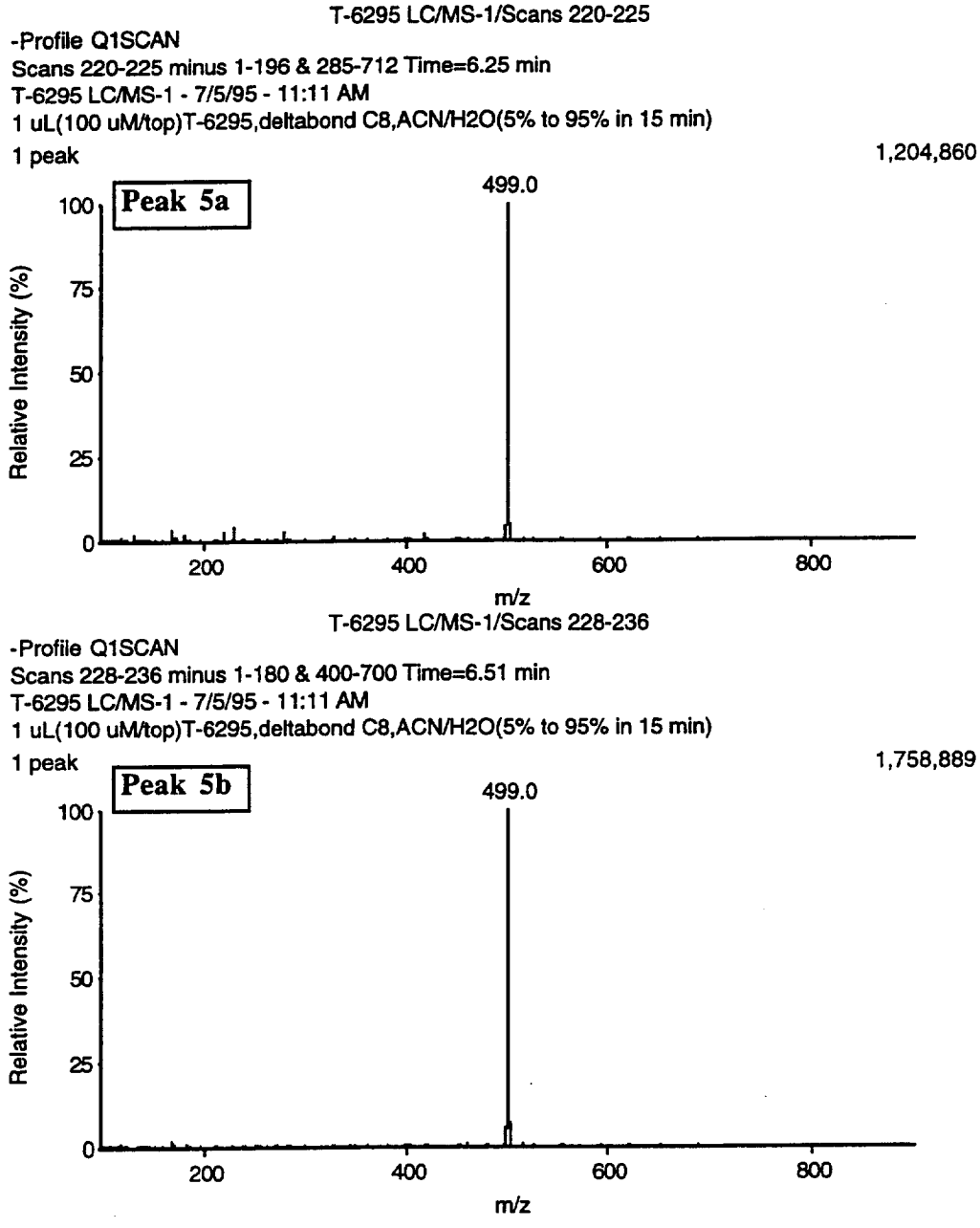

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Figure 10. Full-Scan Mass Spectrum of Peak 6 in Figure 4

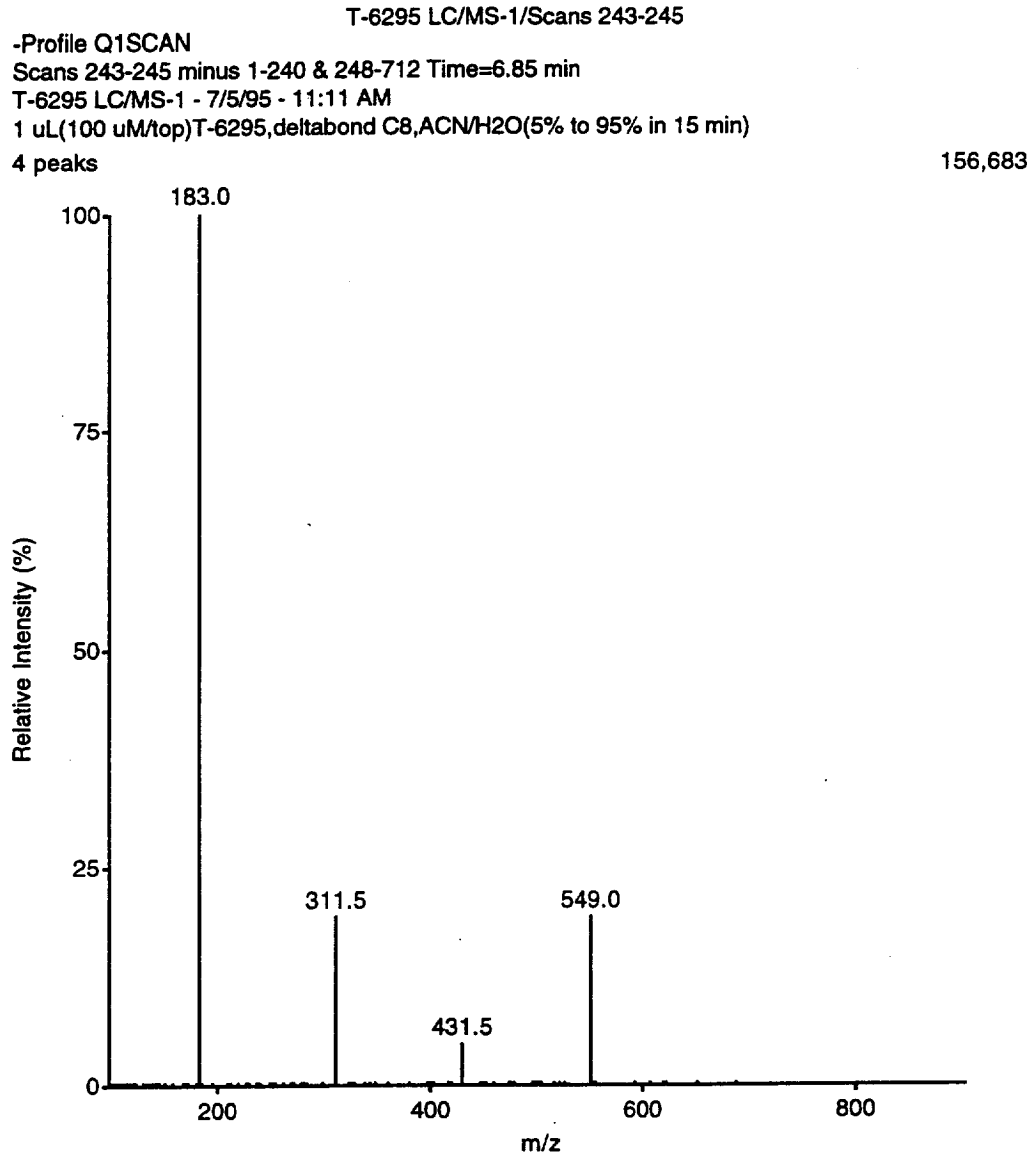

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Figure 11. Full-Scan Mass Spectrum of Peak 7 in Figure 4

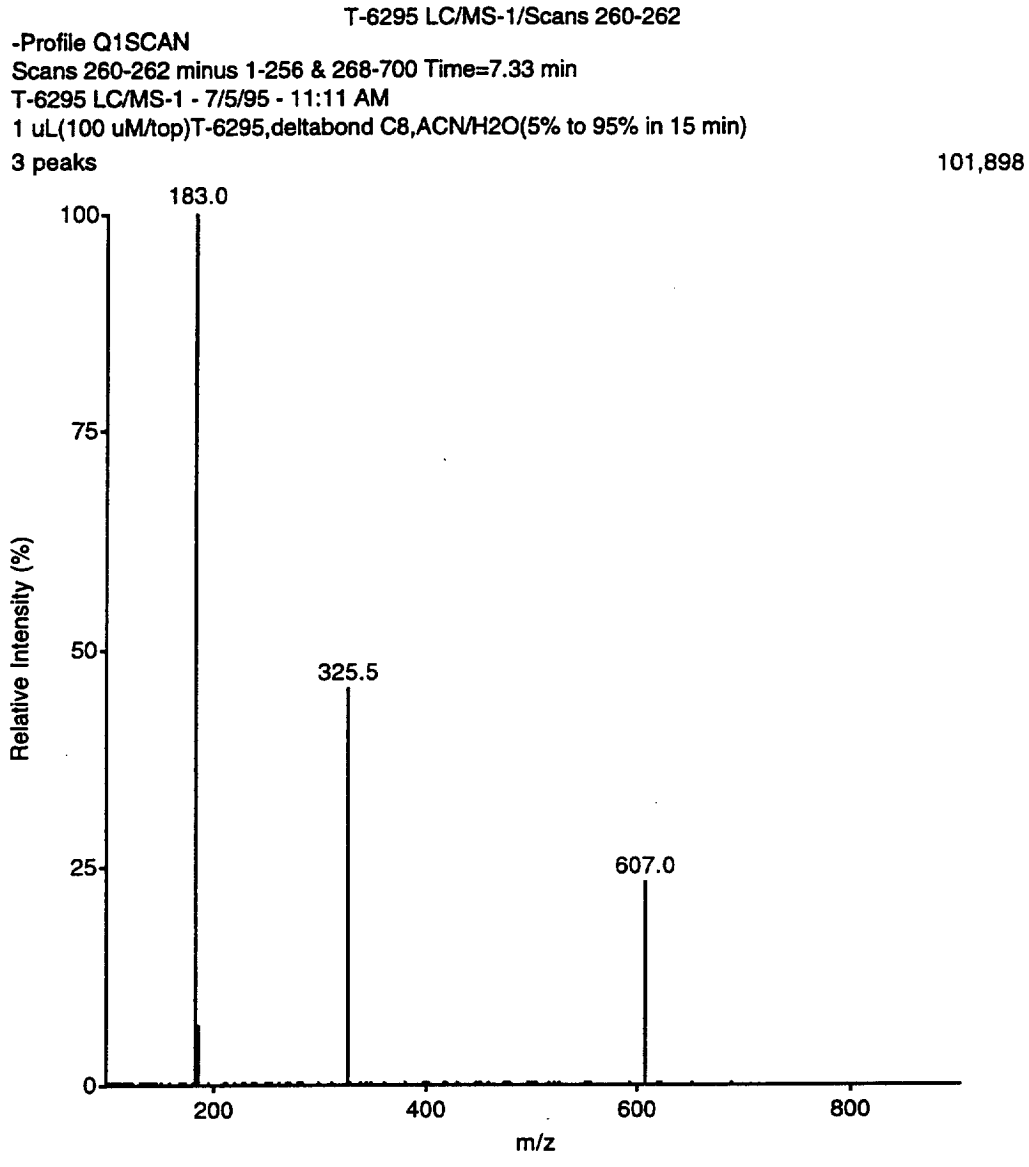

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Figure 12. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Zero Solution of T-6294

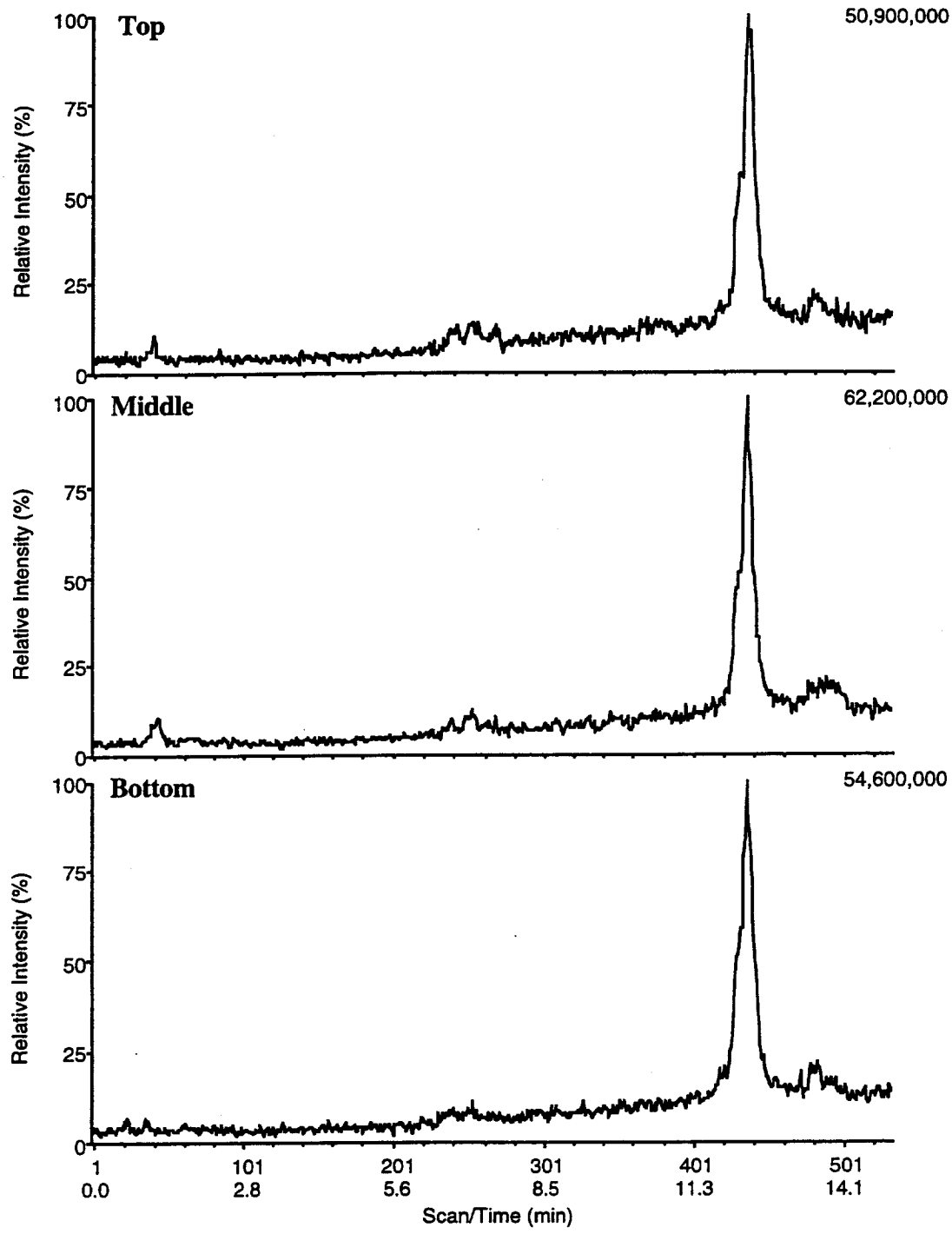

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Figure 13. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Seven Solution of T-6294

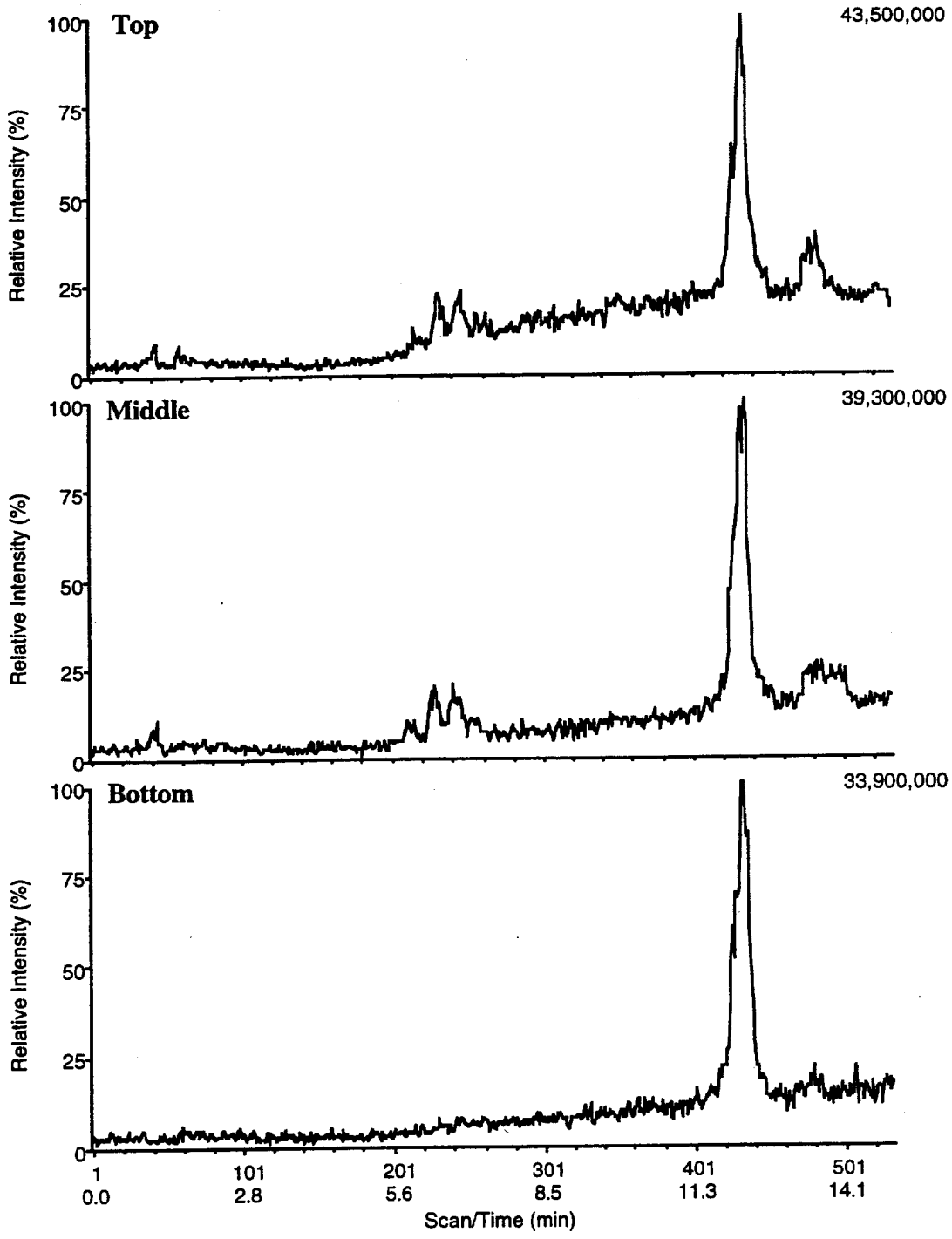

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Figure 14. Extracted Ion Chromatograms for the Injection of 1 µL of Day Seven Top Solution of T-6294

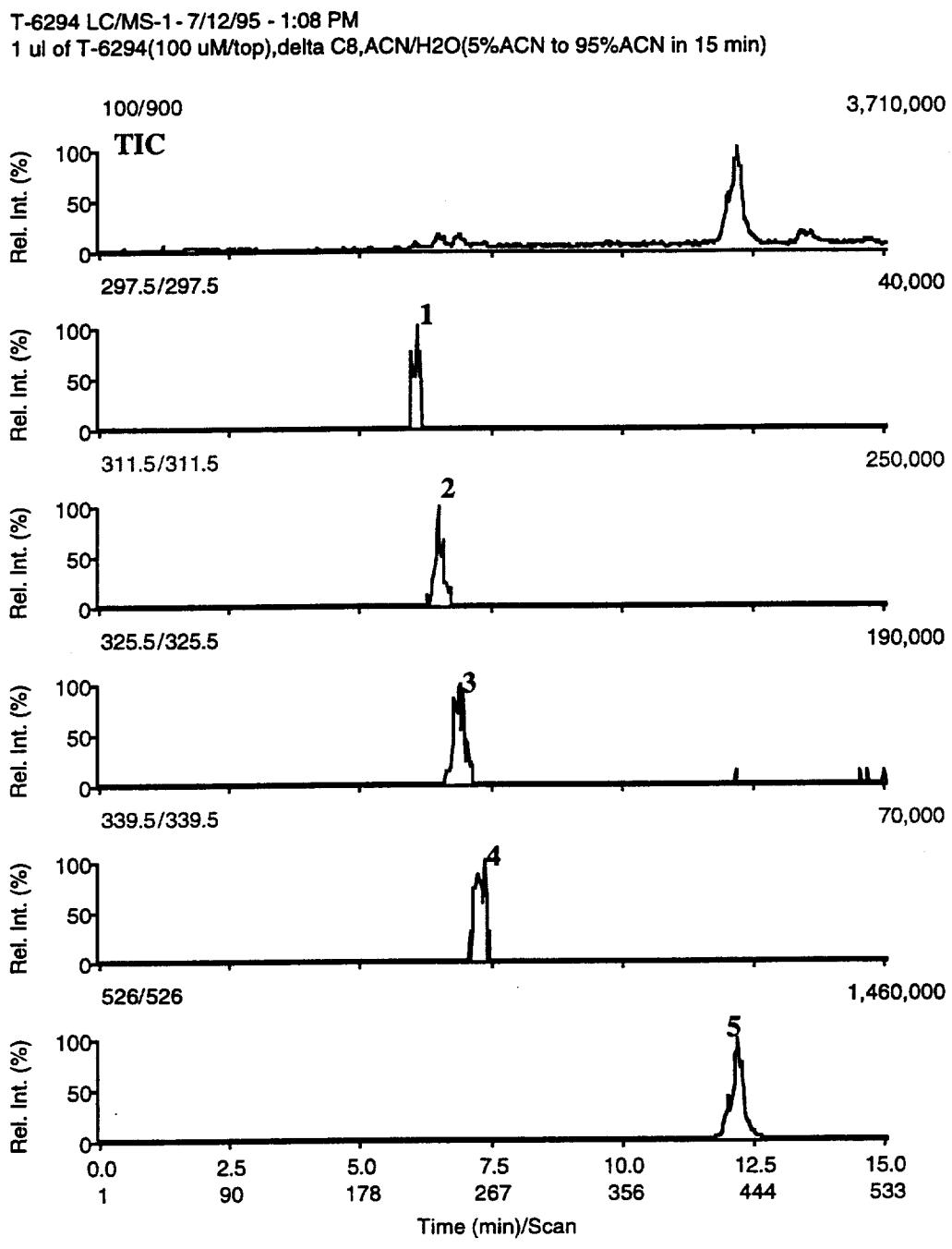

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Figure 15. Full-Scan Mass Spectrum of Peak 1 in Figure 14

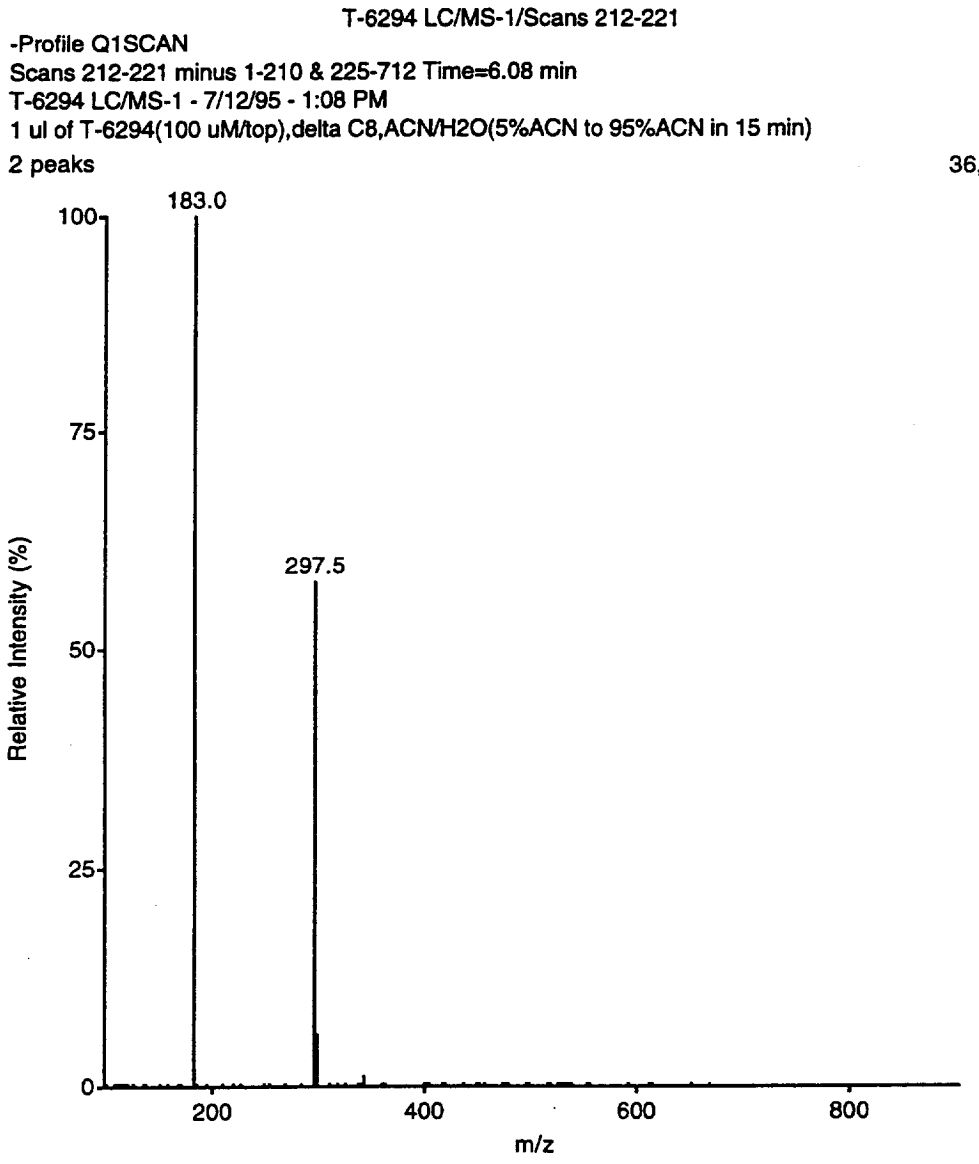

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Figure 16. Full-Scan Mass Spectrum of Peak 2 in Figure 14

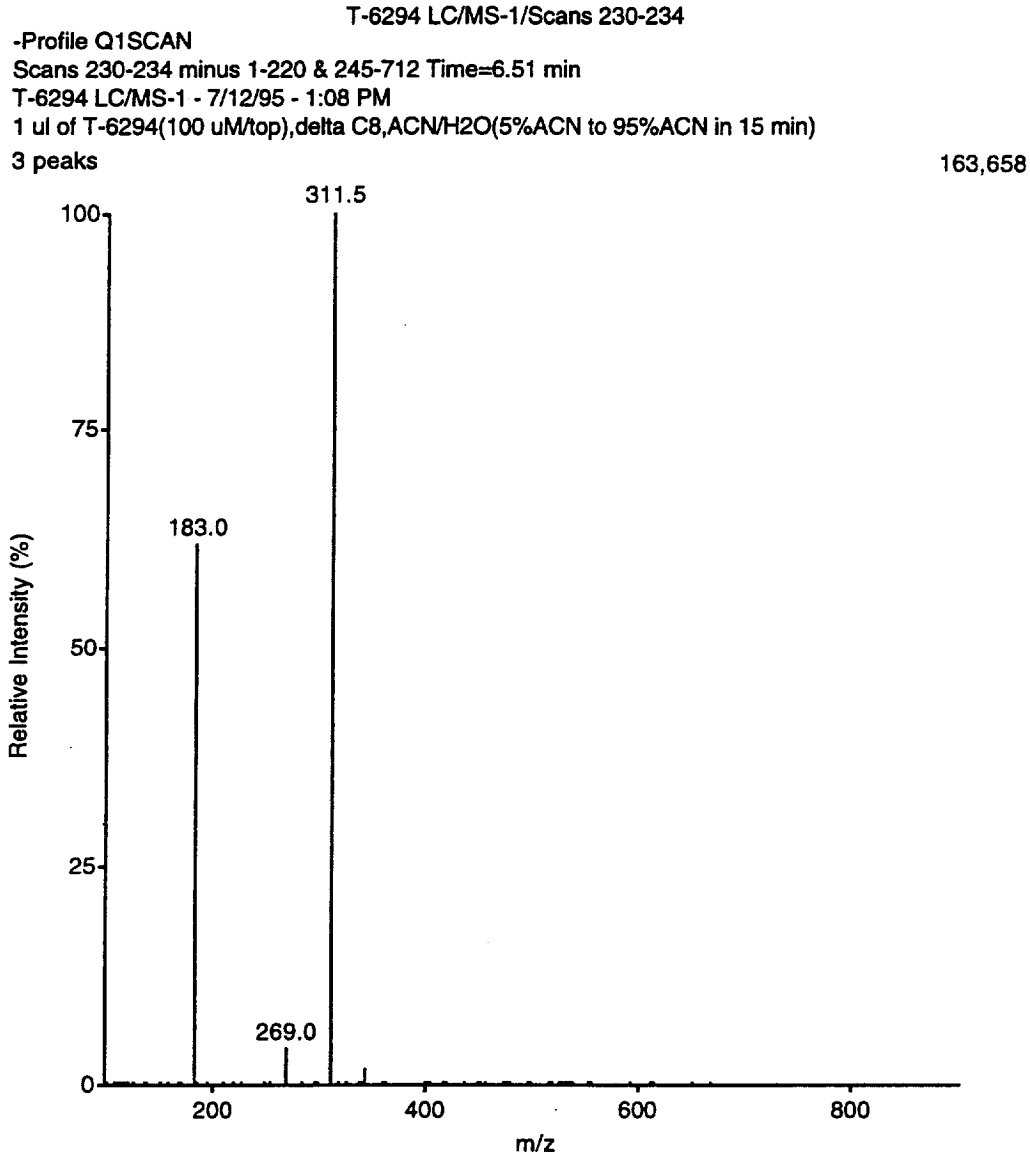

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Figure 17. Full-Scan Mass Spectrum of Peak 3 in Figure 14

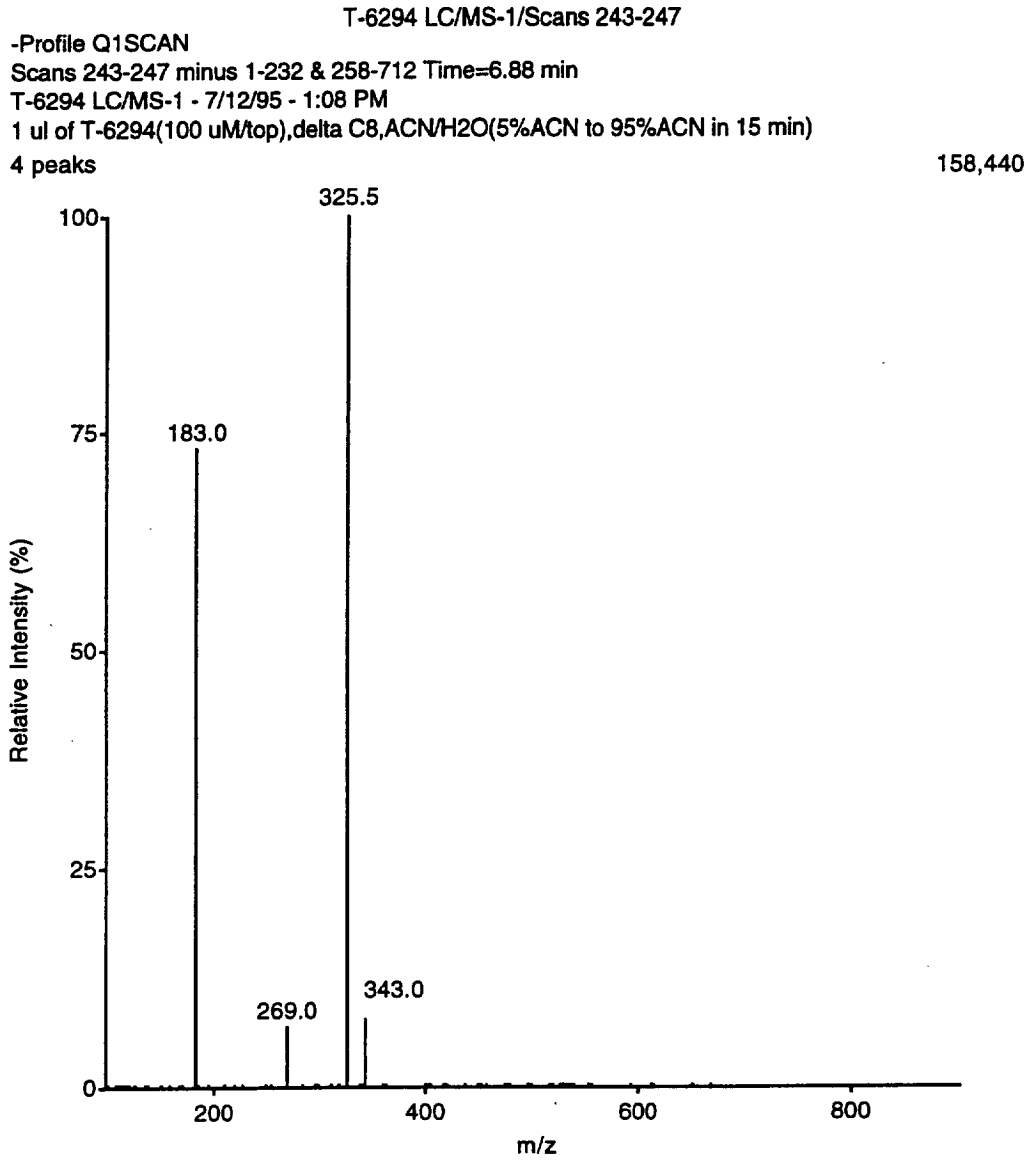

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Figure 18. Full-Scan Mass Spectrum of Peak 4 in Figure 14

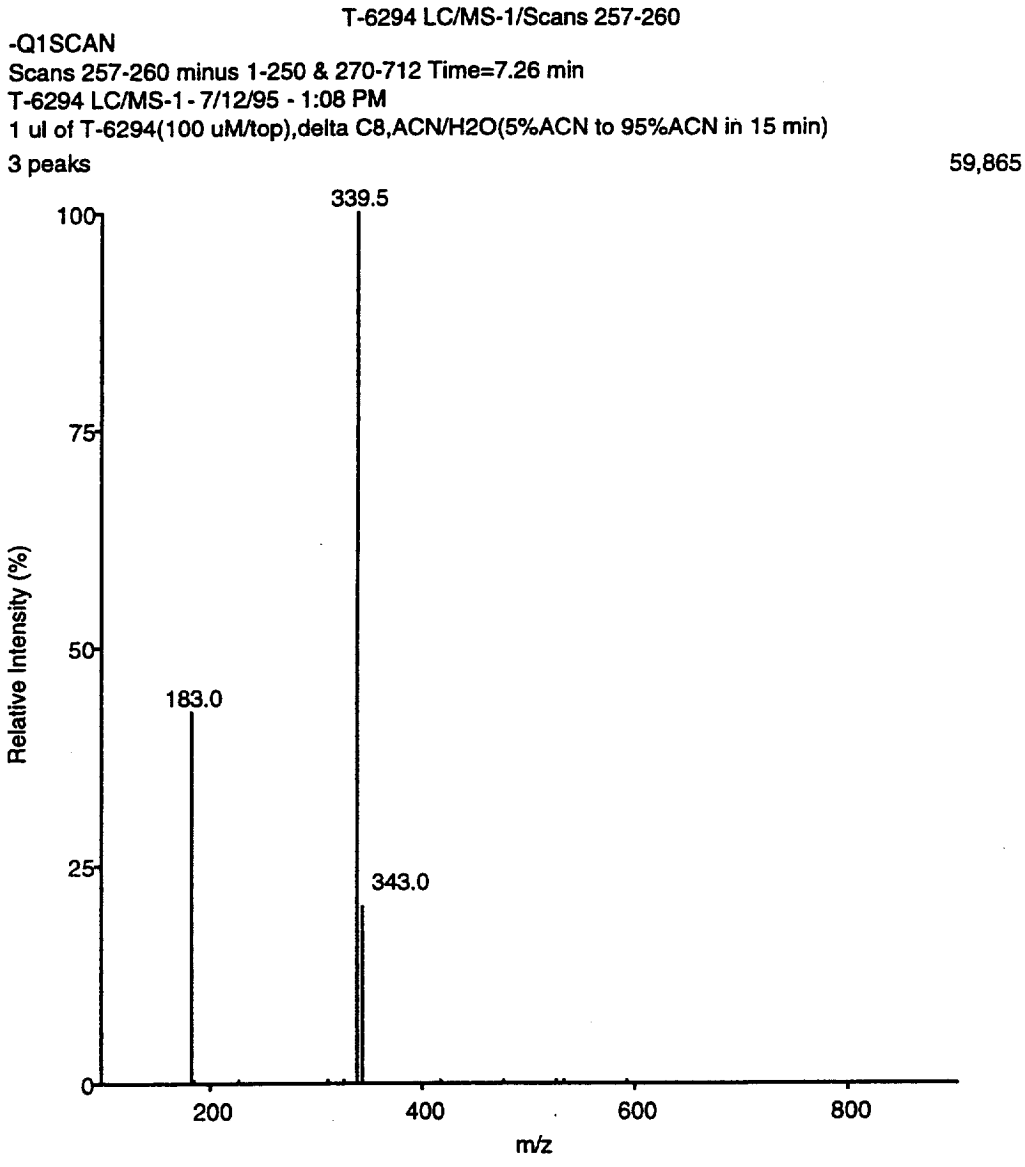

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Figure 19. Full-Scan Mass Spectrum of Peak 5 in Figure 14

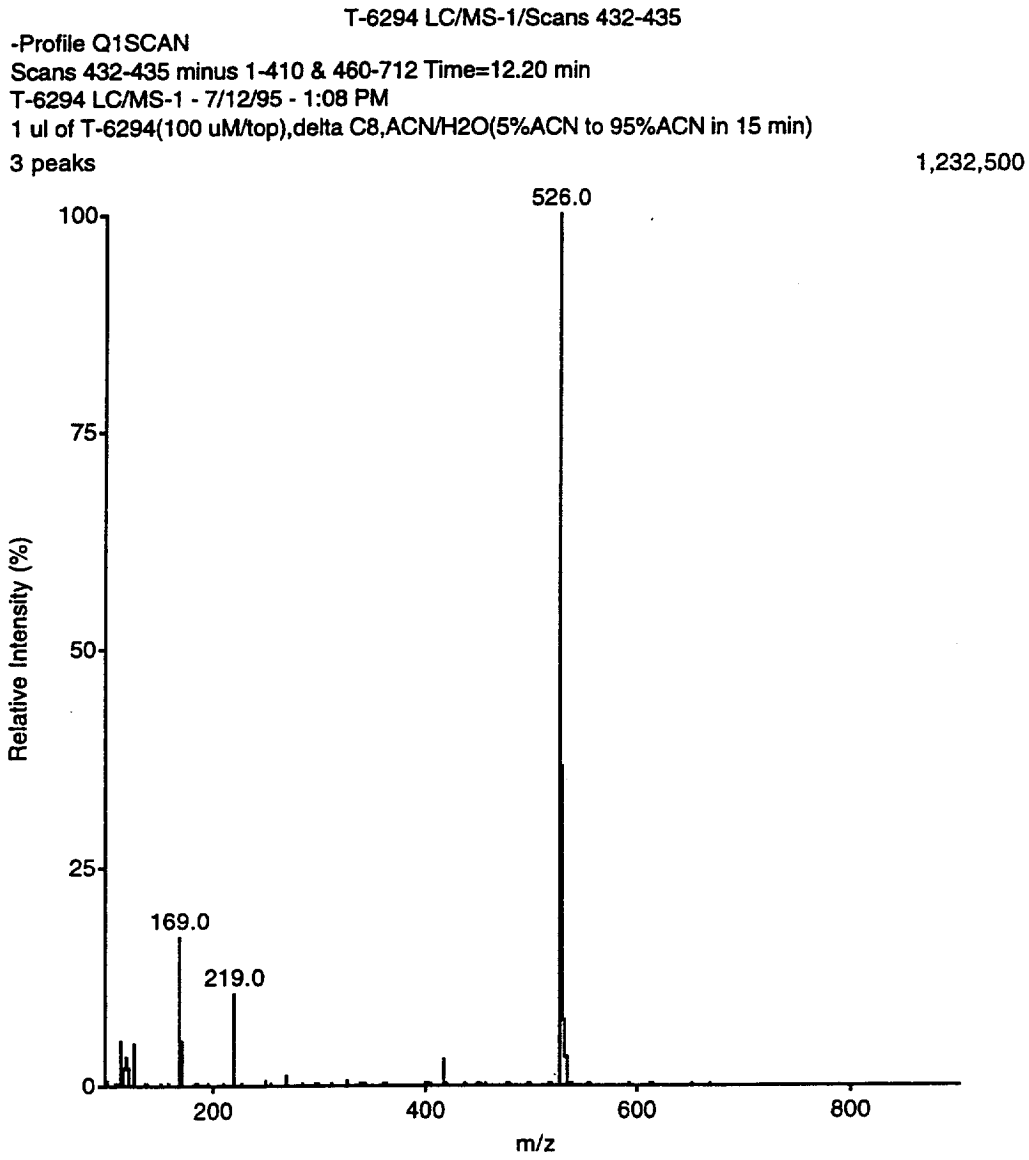

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Figure 20. Total Ion Chromatogram (TIC) for the Injection of 1 µL of Water

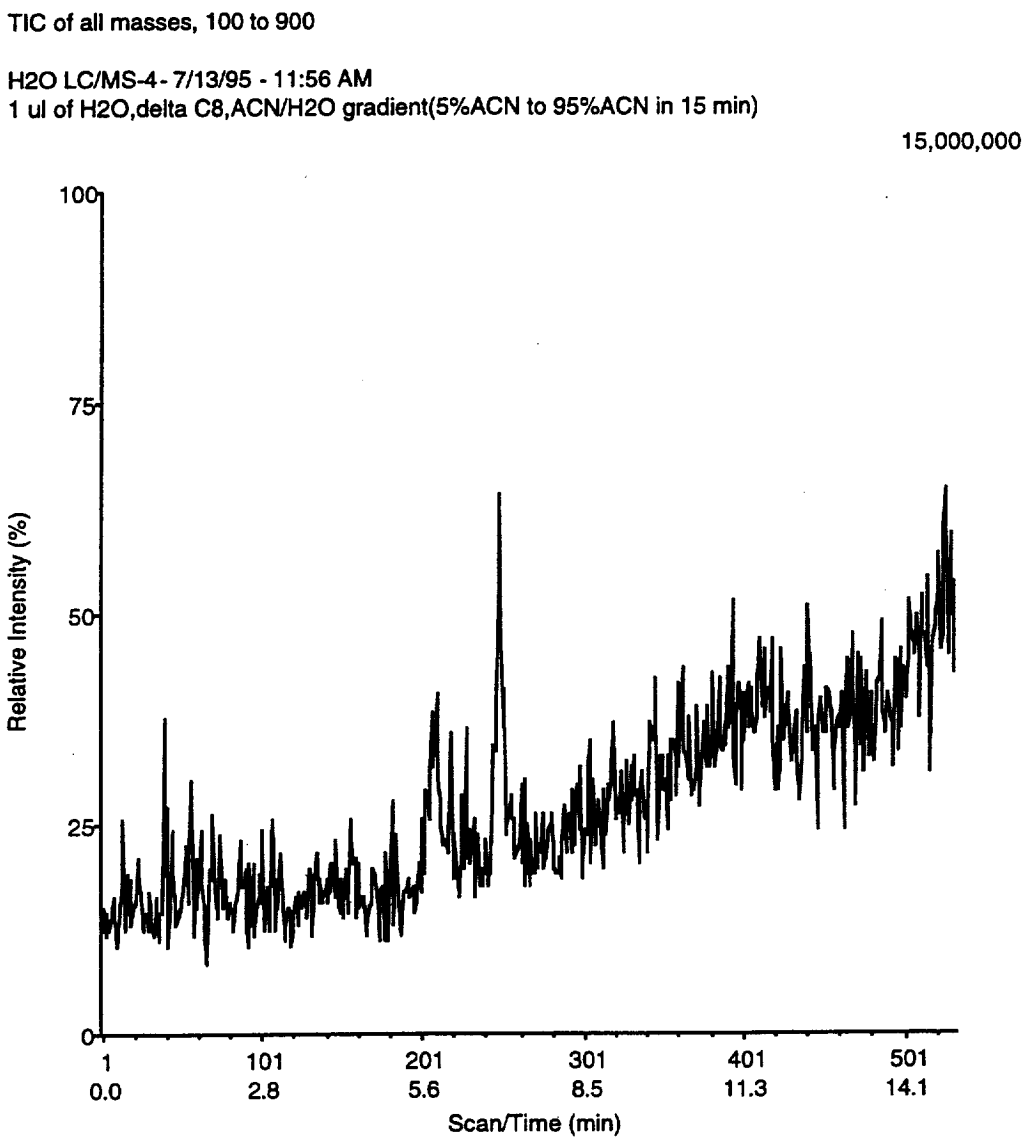

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Figure 21. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Zero Solution of T-6293

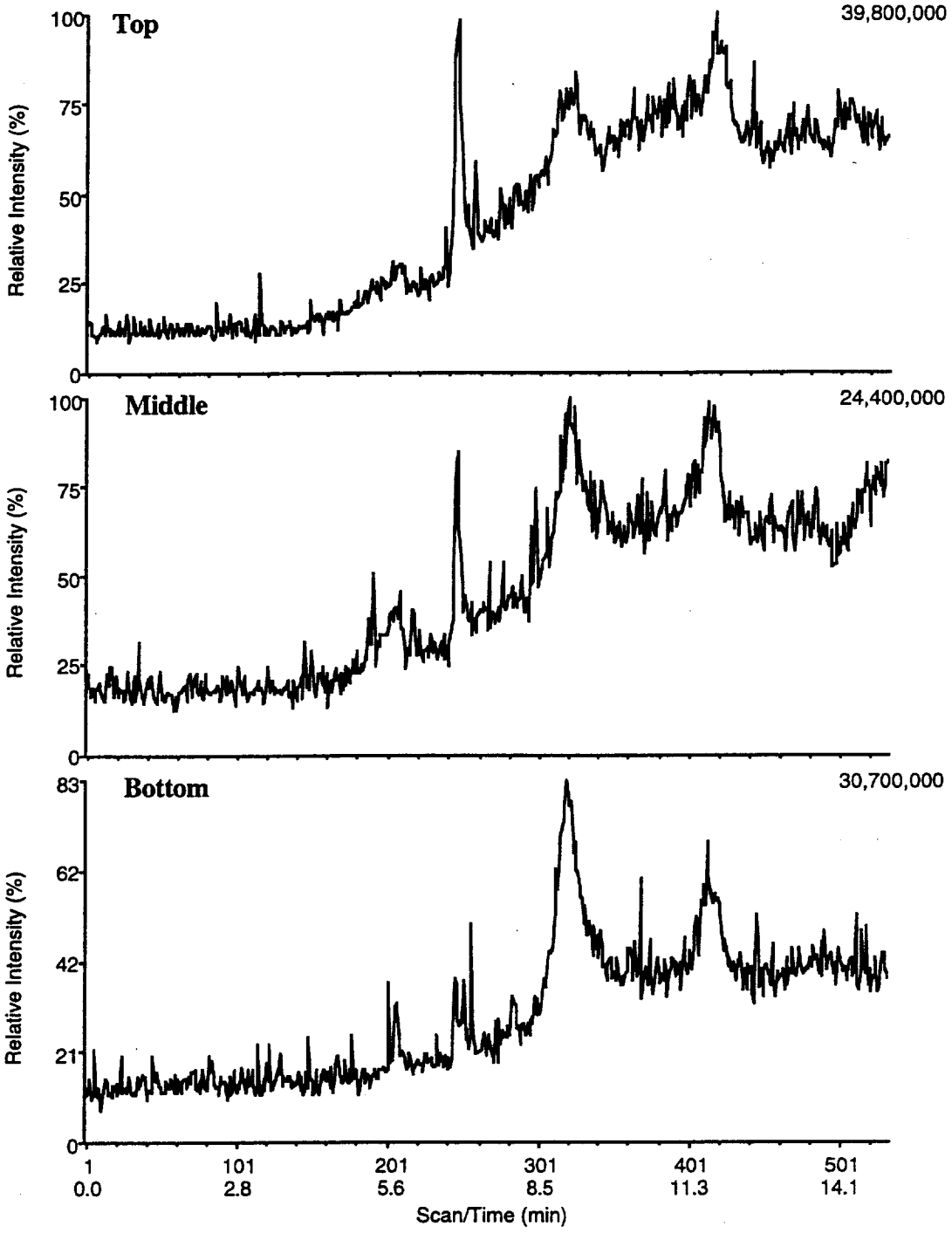

Figure 22. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Seven Solution of T-6293

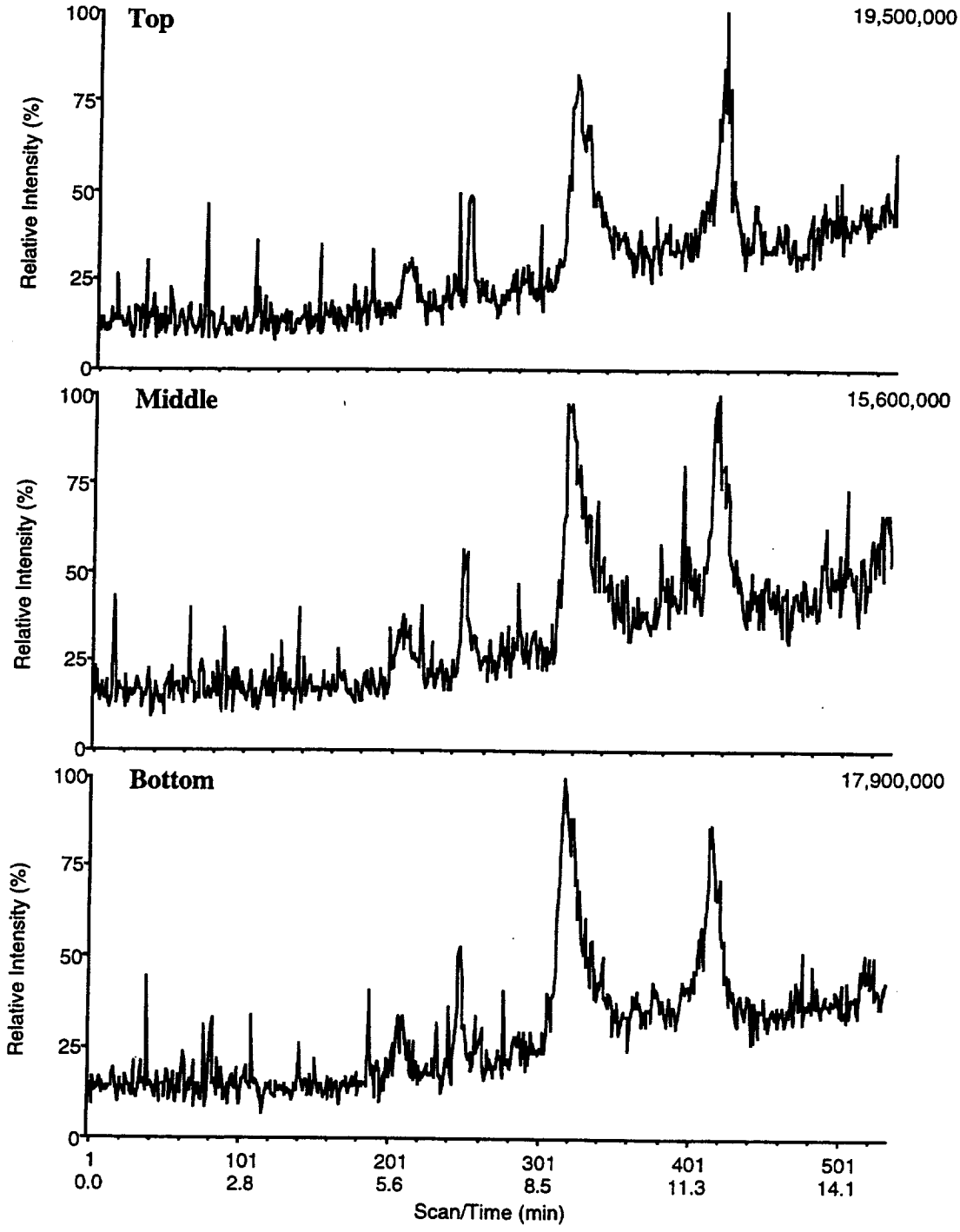

Figure 23. Extracted Ion Chromatograms for the Injection of 1 μL of Day Seven Bottom Solution of T-6293

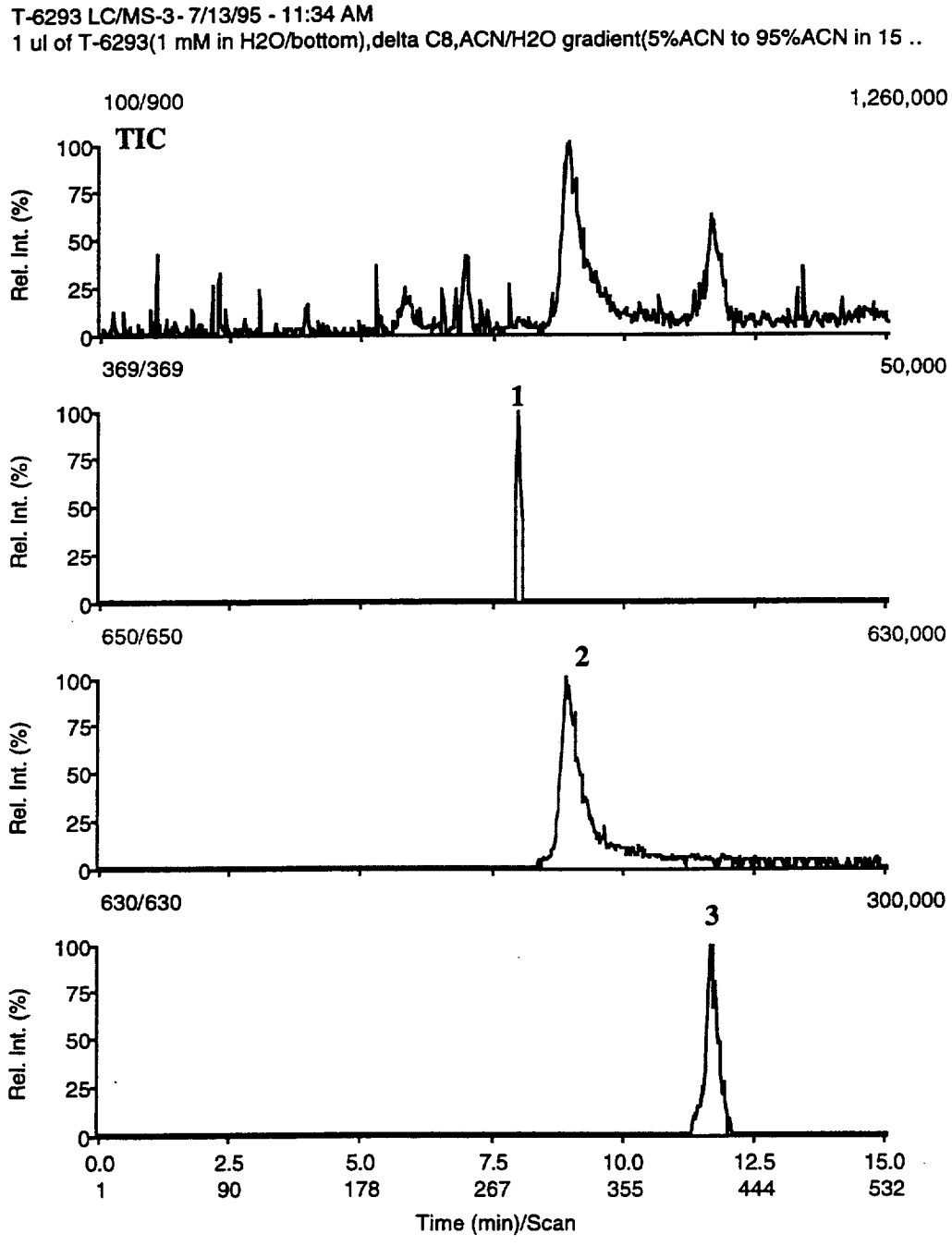

Figure 24. Full-Scan Mass Spectrum of Peak 1 in Figure 23

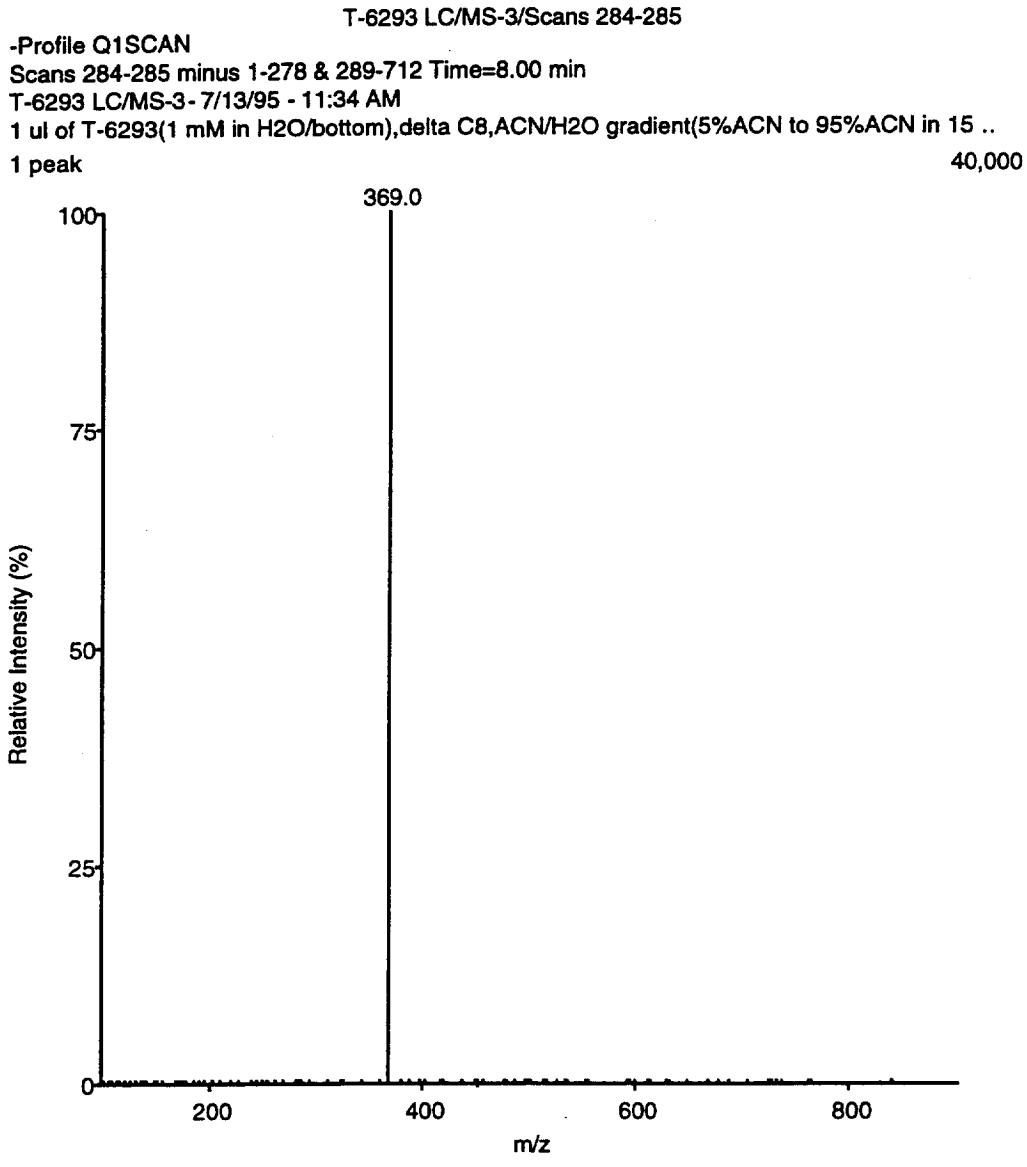

Figure 25. Full-Scan Mass Spectrum of Peak 2 in Figure 23

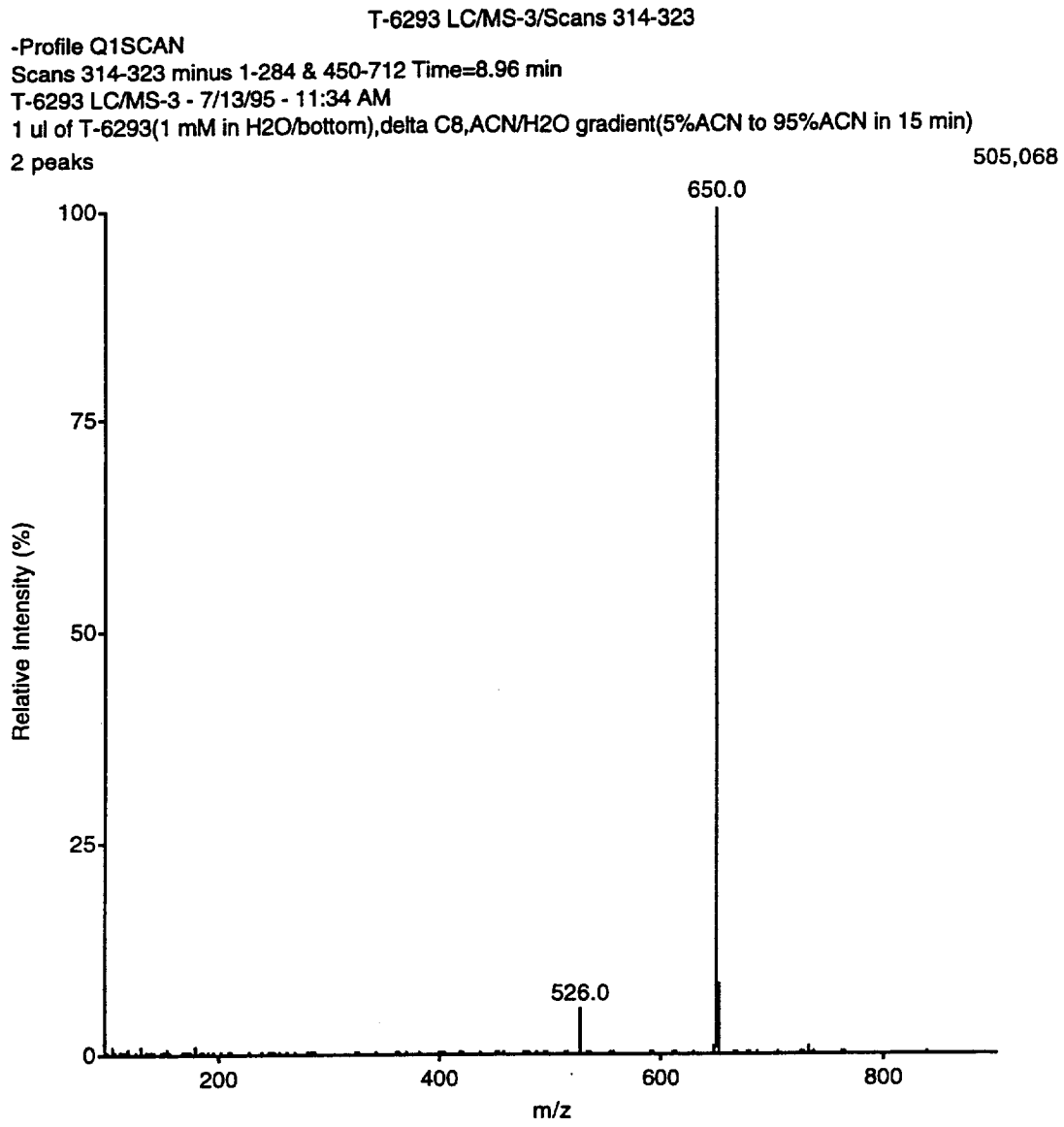

Figure 26. Full-Scan Mass Spectrum of Peak 3 in Figure 23

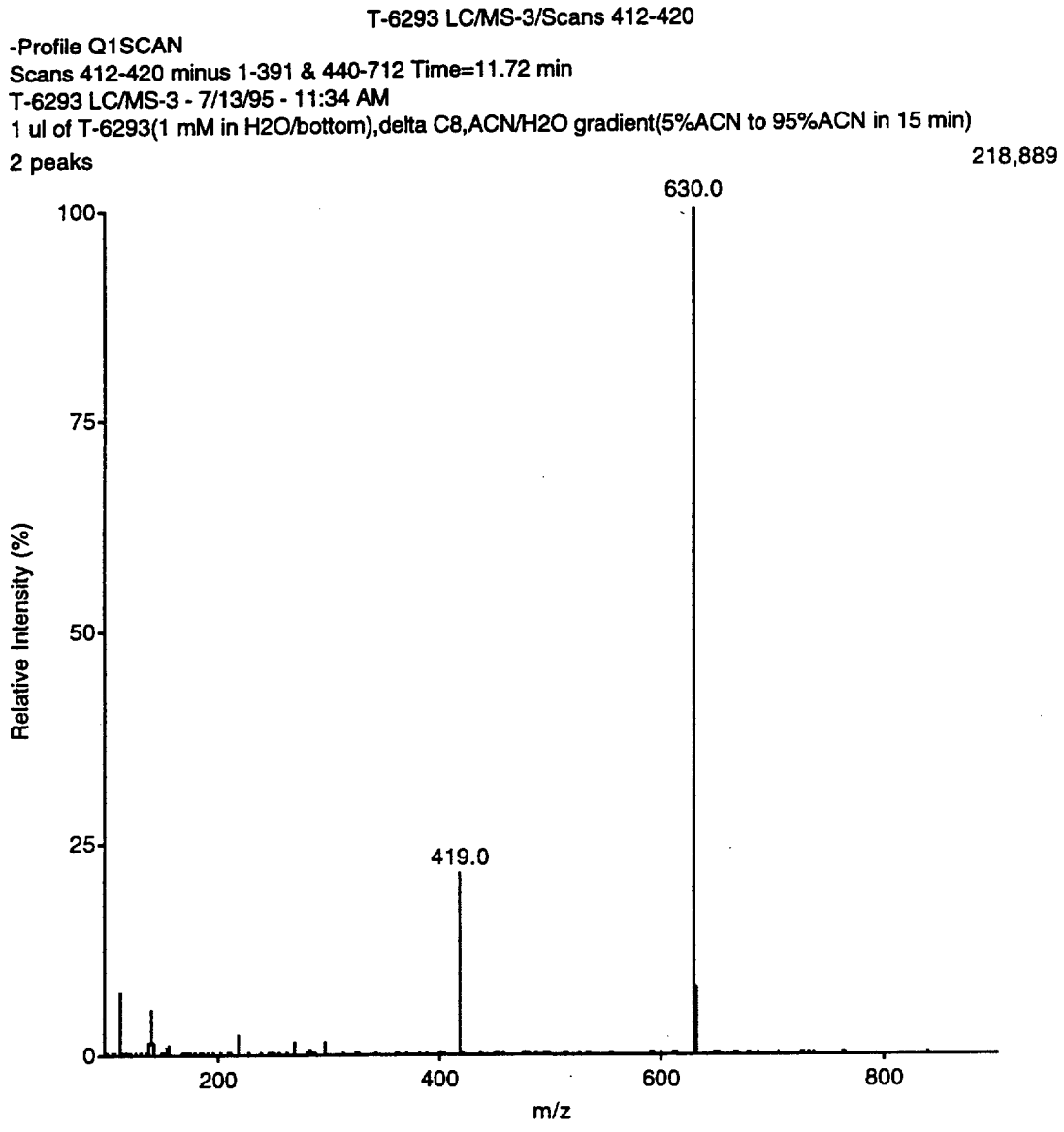

Figure 27. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Zero Solution of Fose Amide

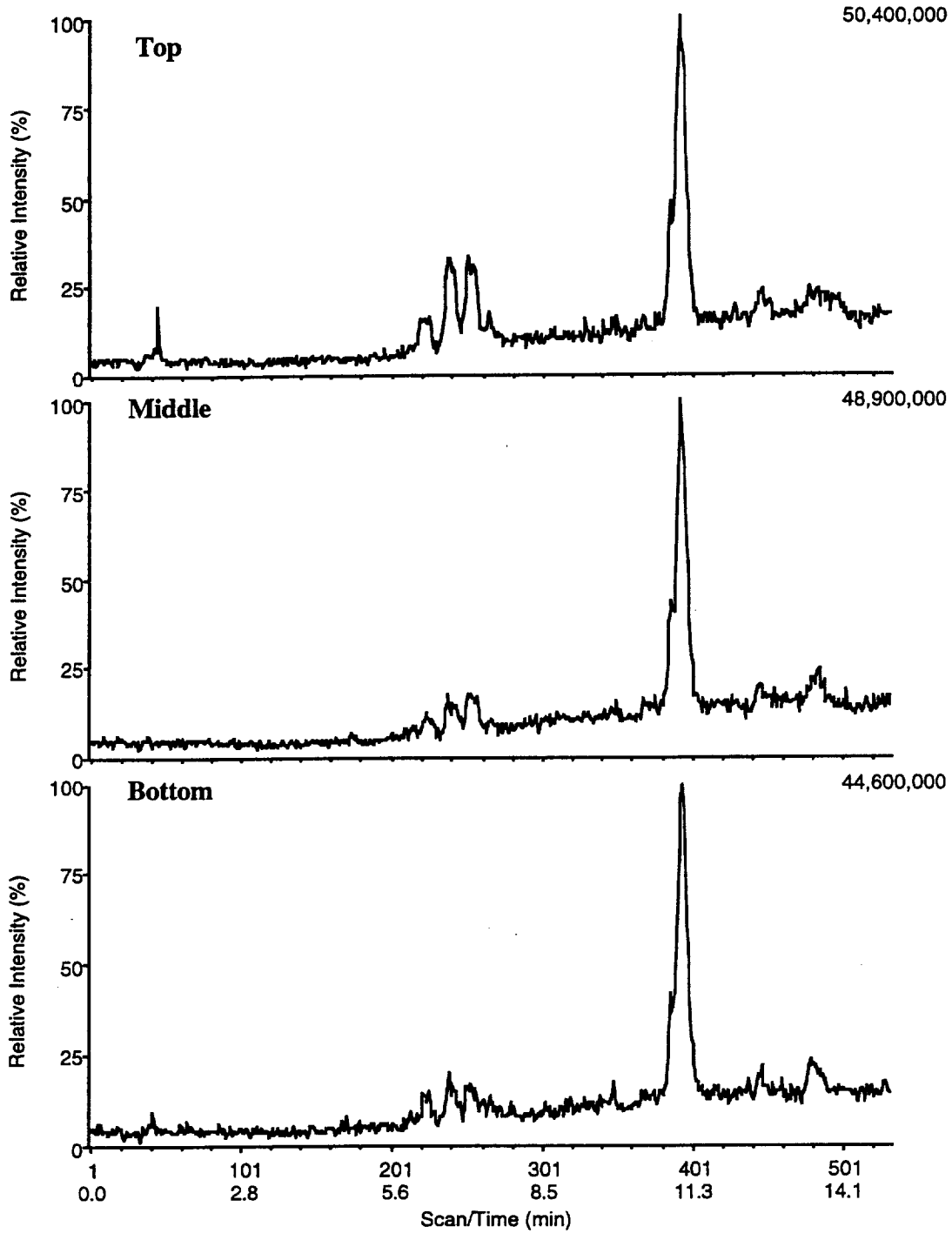

Figure 28. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Seven Solution of Fose Amide

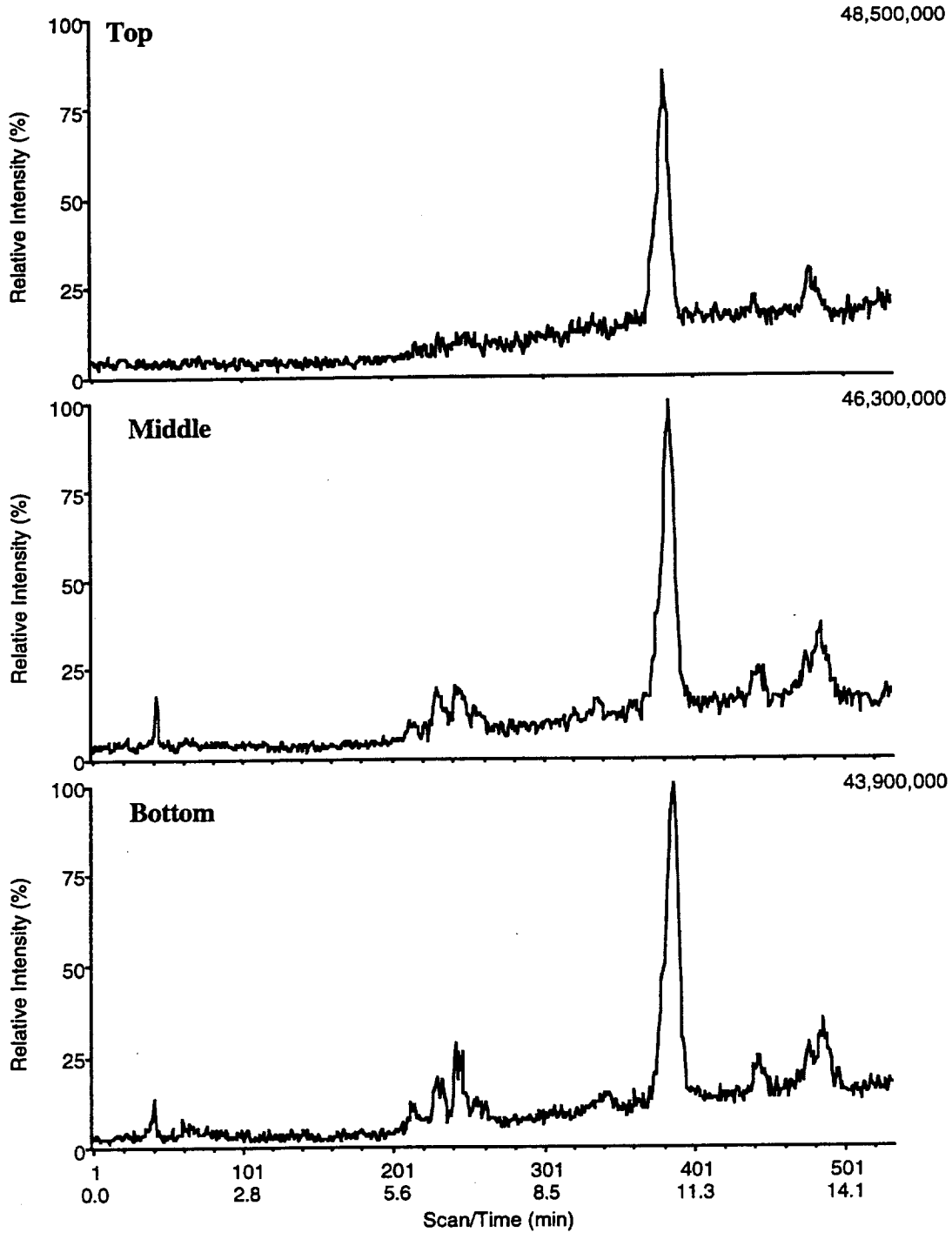

Figure 29. Extracted Ion Chromatograms for the Injection of 1 µL of Day Zero Top Solution of Fose Amide

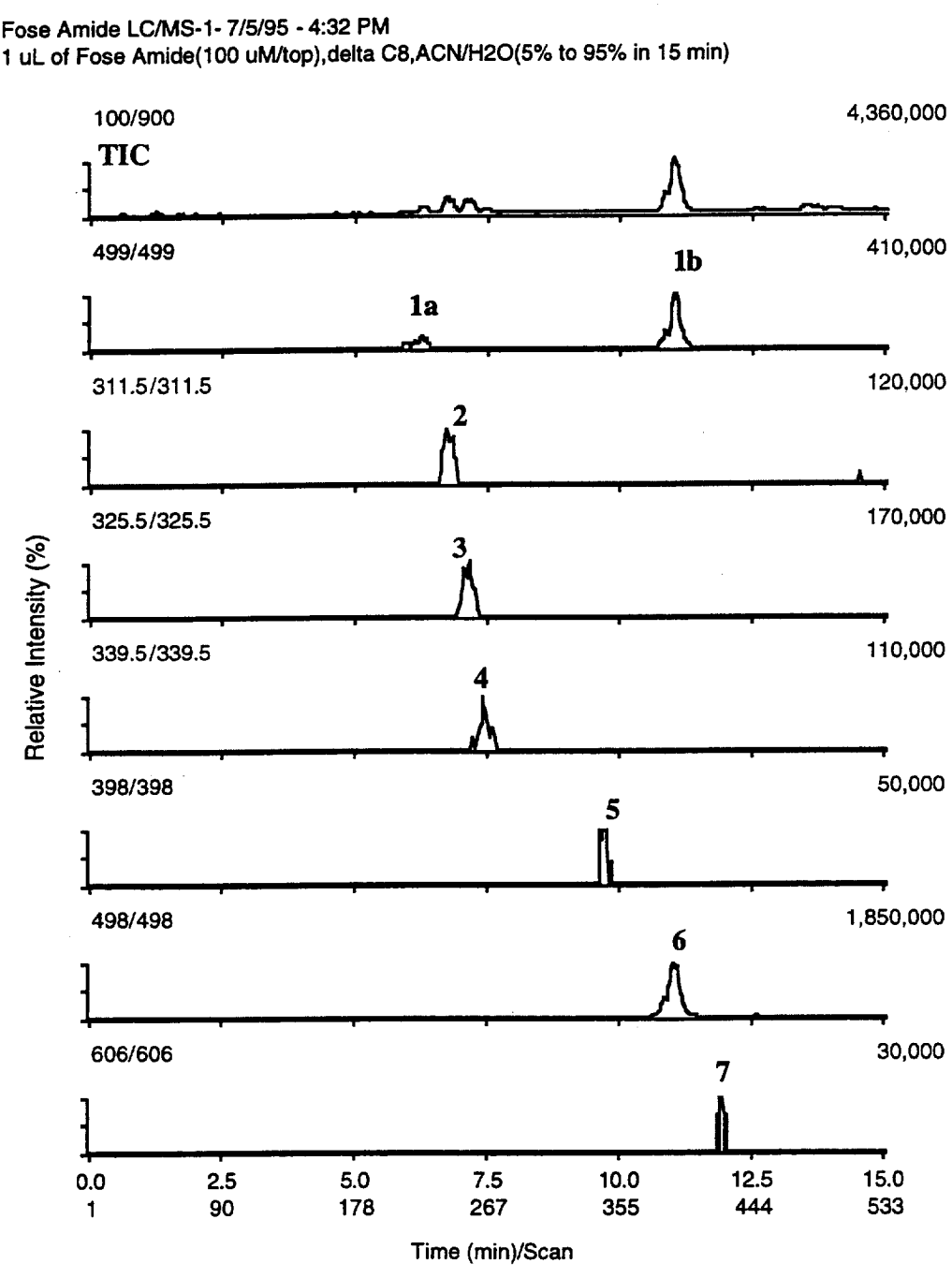

Figure 30. Full-Scan Mass Spectra of Peaks 1a and 1b in Figure 29

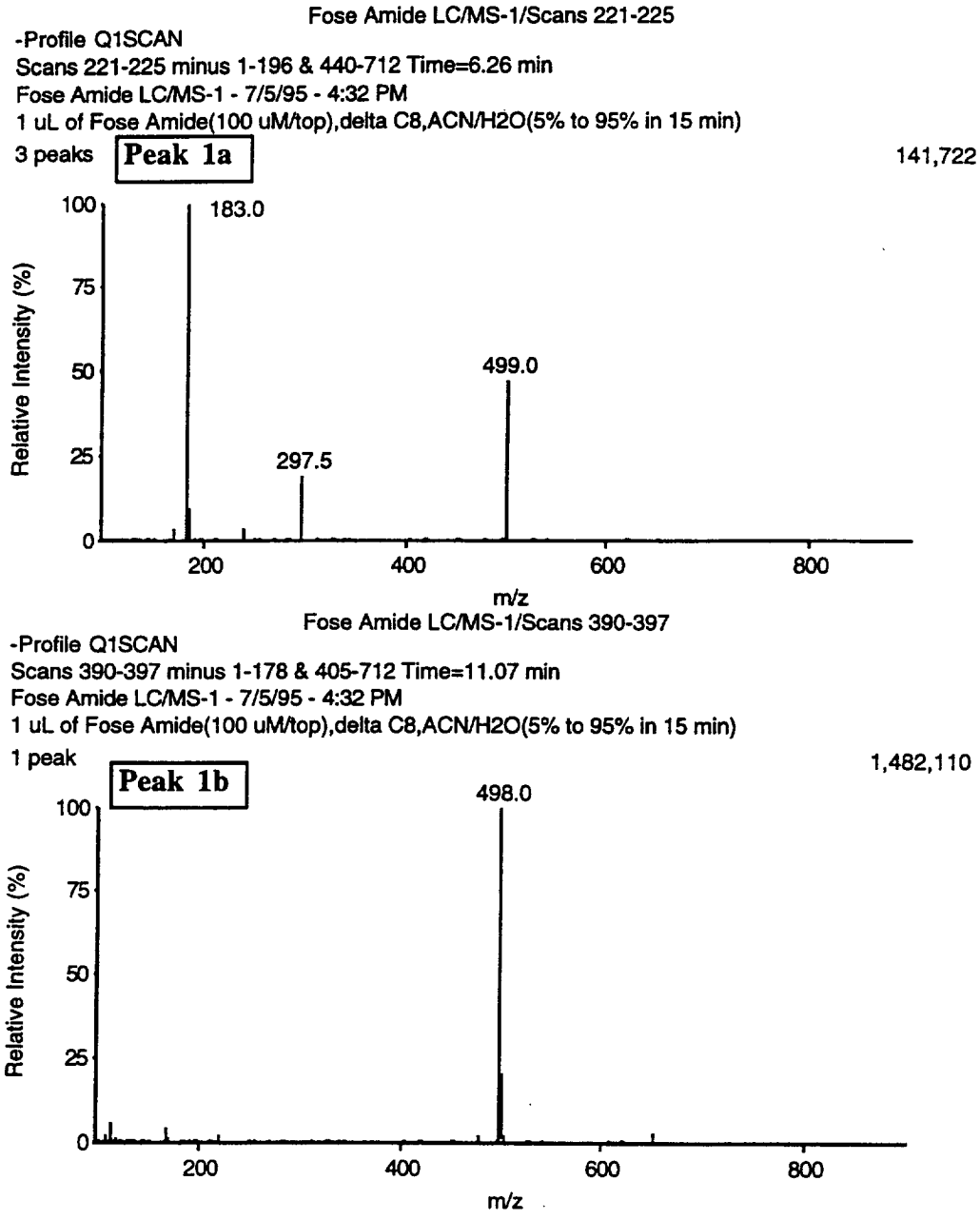

Figure 31. Full-Scan Mass Spectrum of Peak 2 in Figure 29

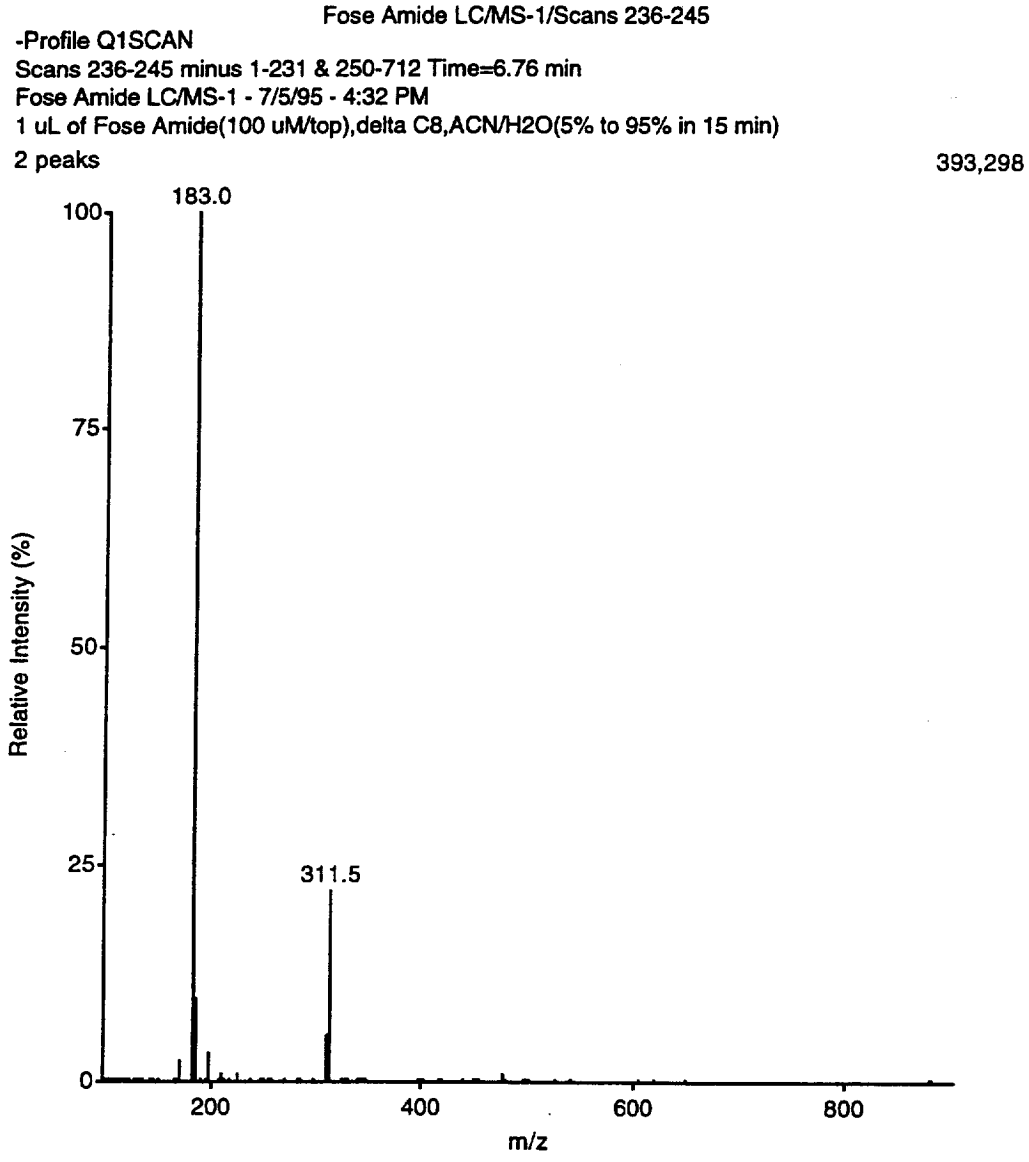

Figure 32. Full-Scan Mass Spectrum of Peak 3 in Figure 29

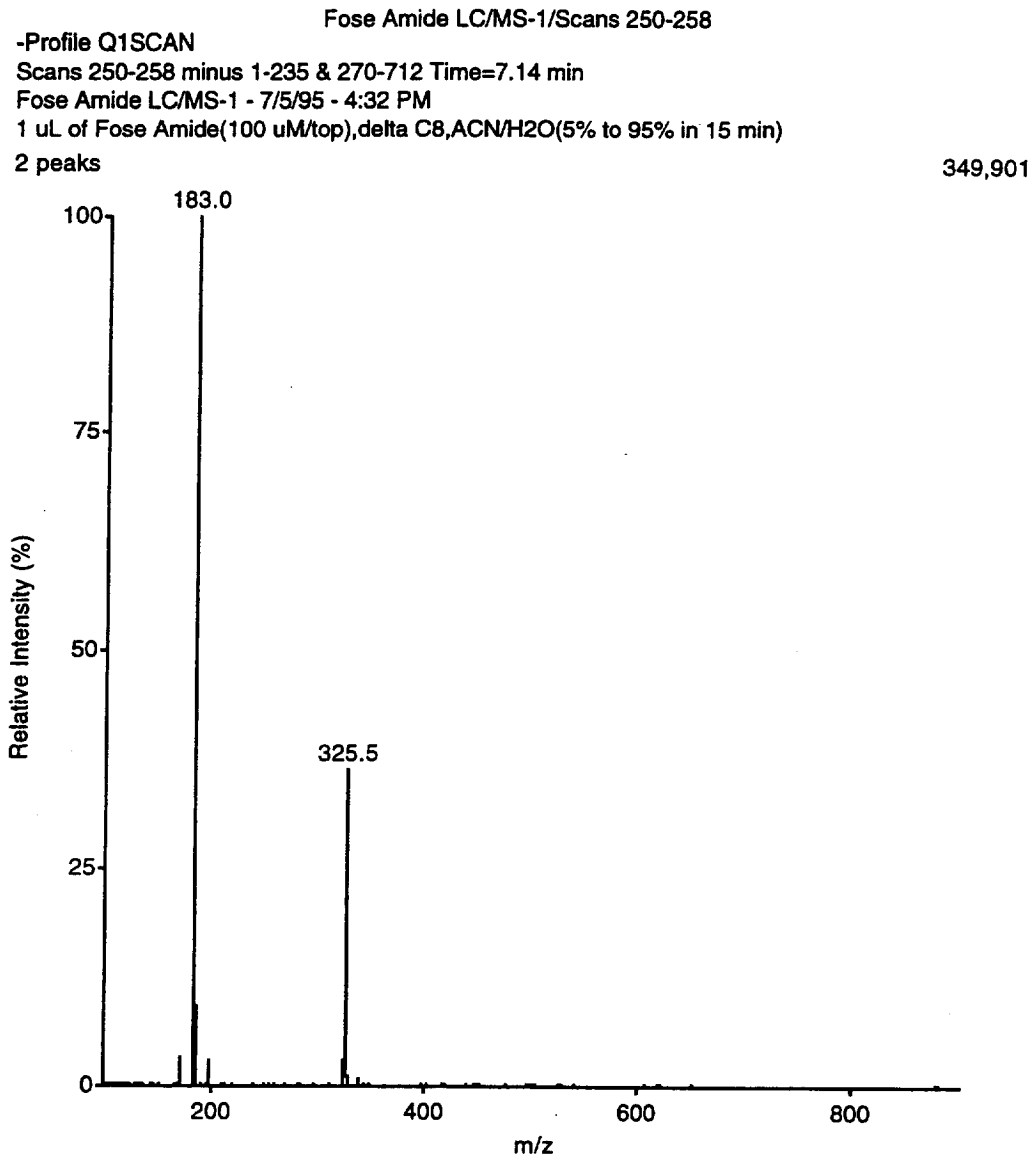

Figure 33. Full-Scan Mass Spectrum of Peak 4 in Figure 29

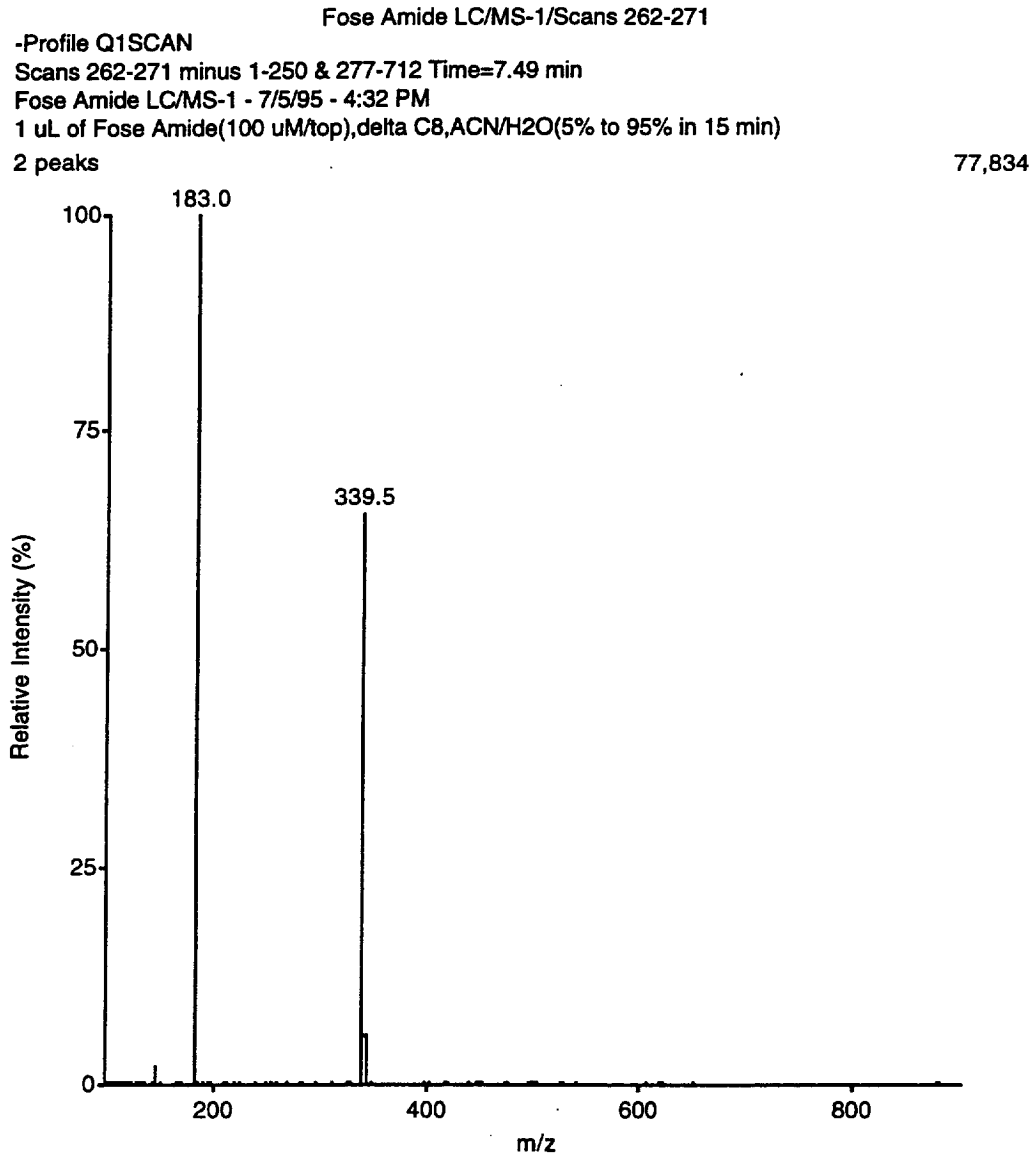

Figure 34. Full-Scan Mass Spectrum of Peak 5 in Figure 29

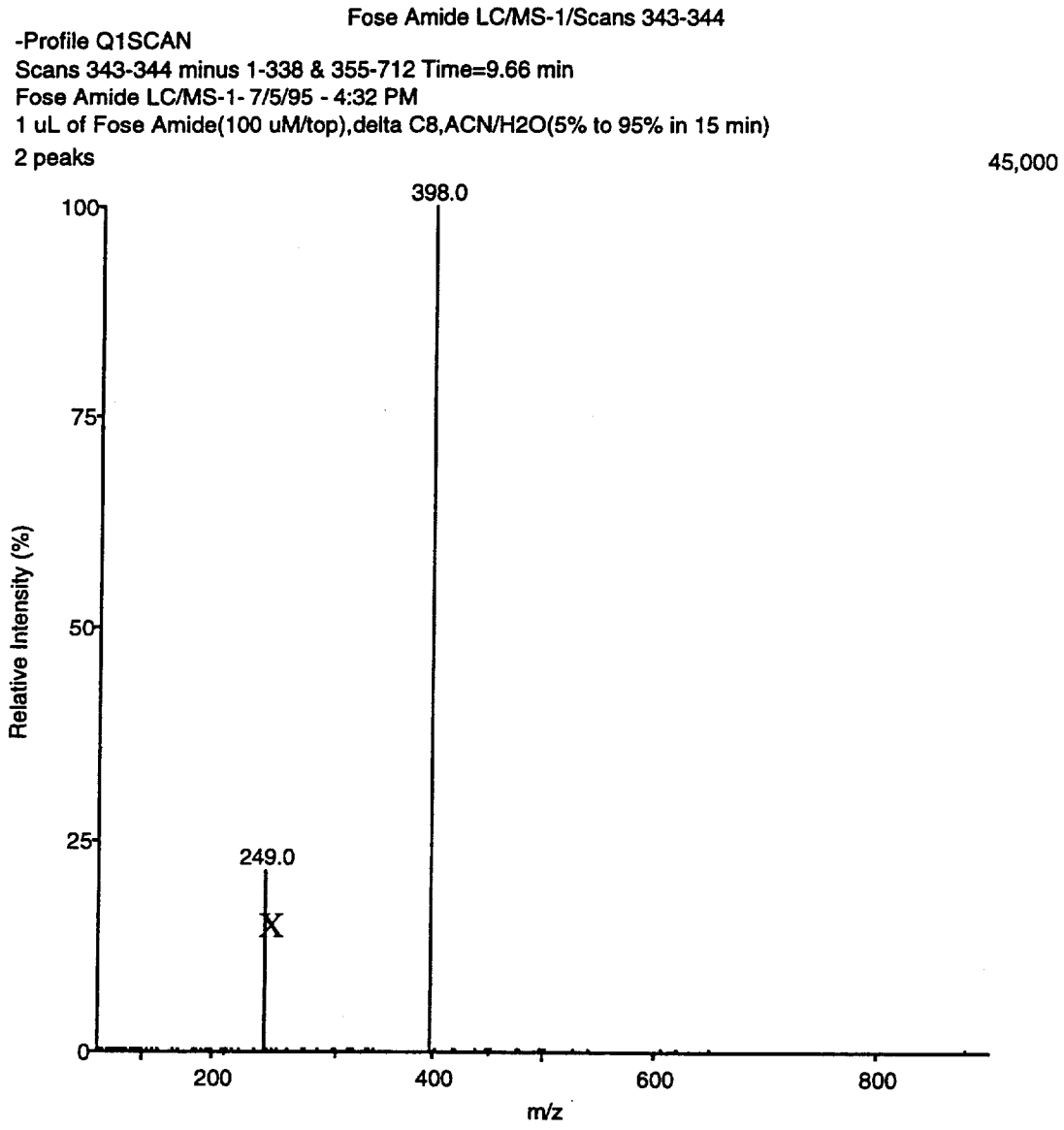

Figure 35. Full-Scan Mass Spectrum of Peak 6 in Figure 29

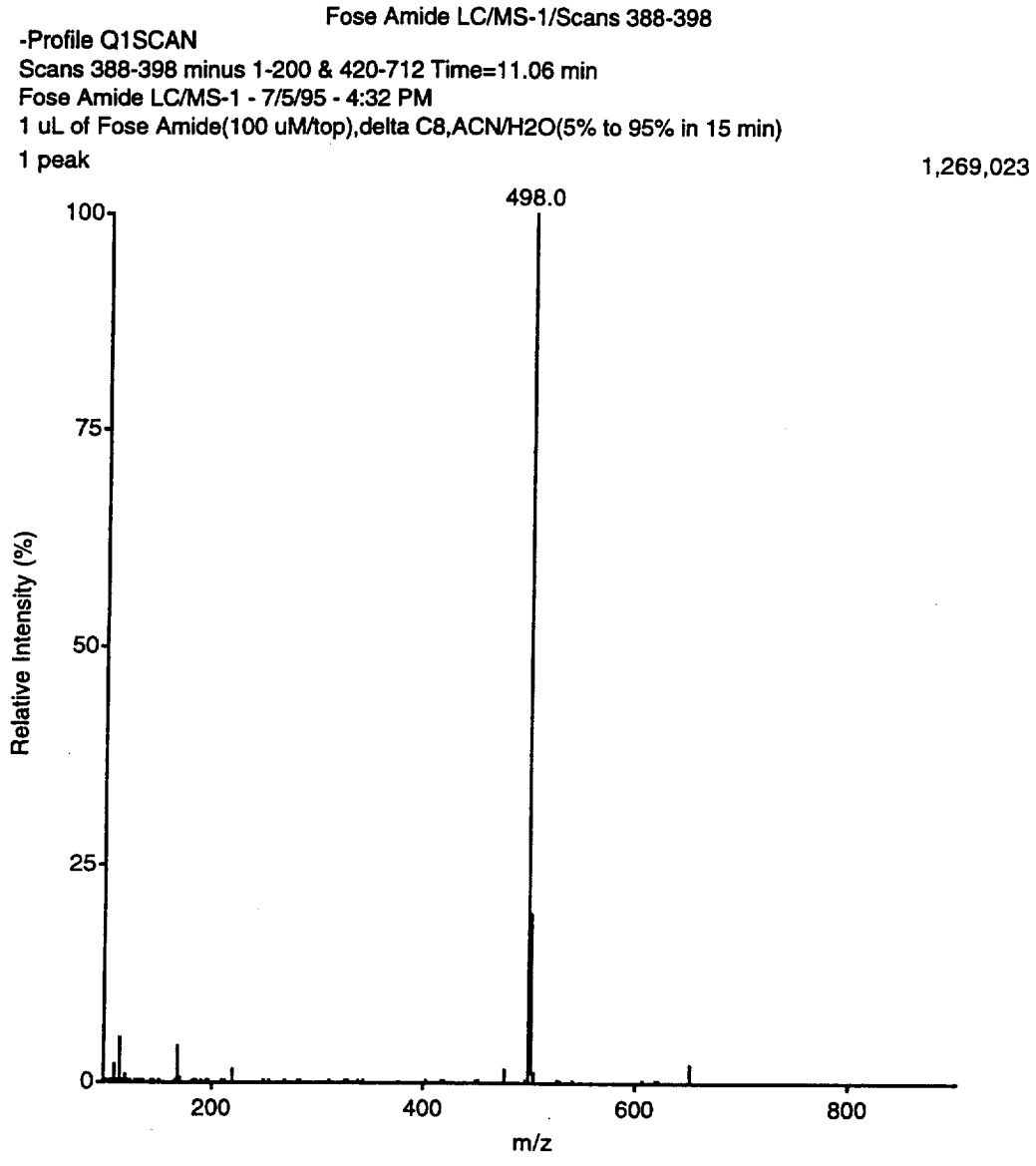

Figure 36. Full-Scan Mass Spectrum of Peak 7 in Figure 29

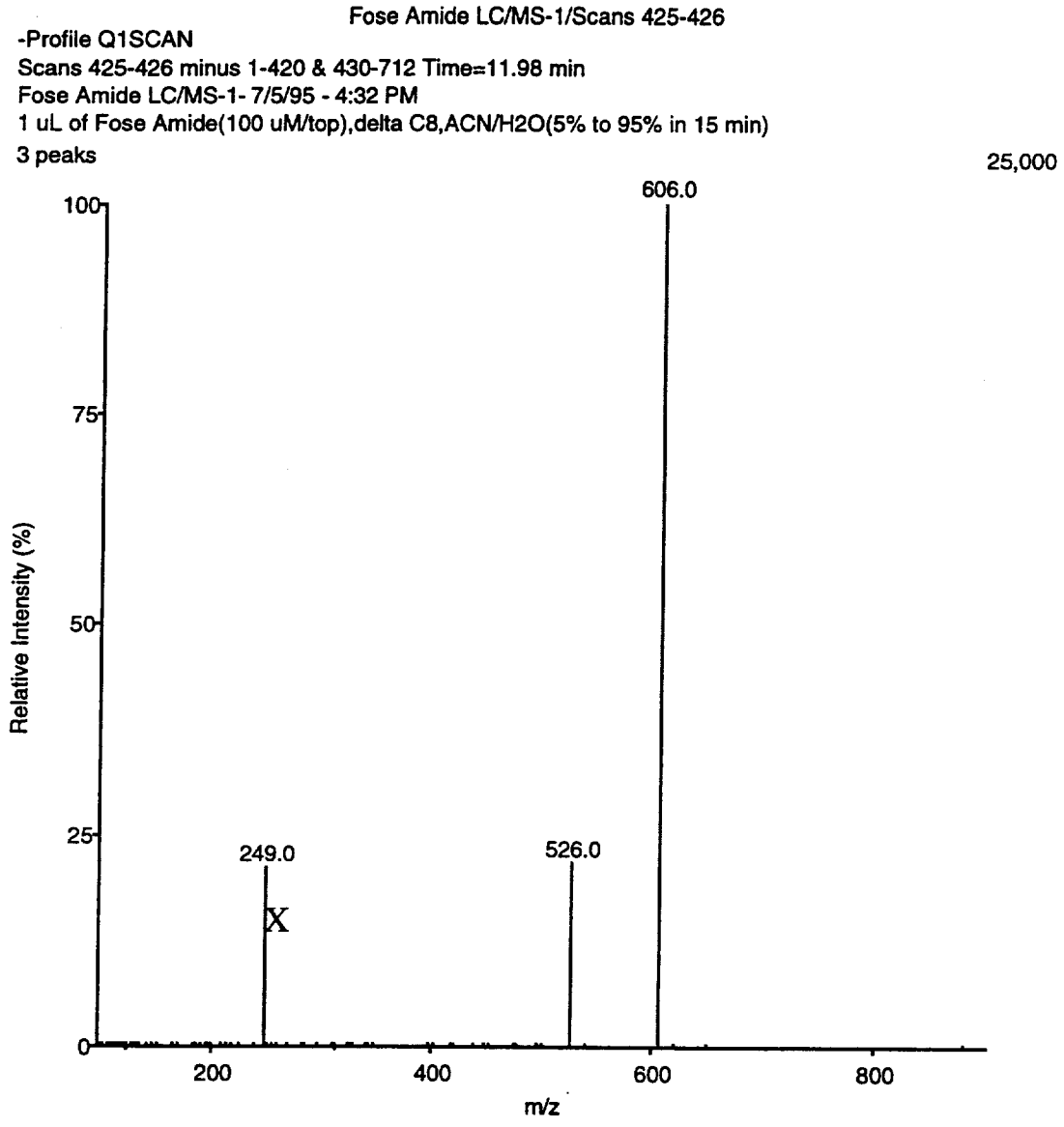

Figure 37. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Zero Solution of Perfluorooctanamide

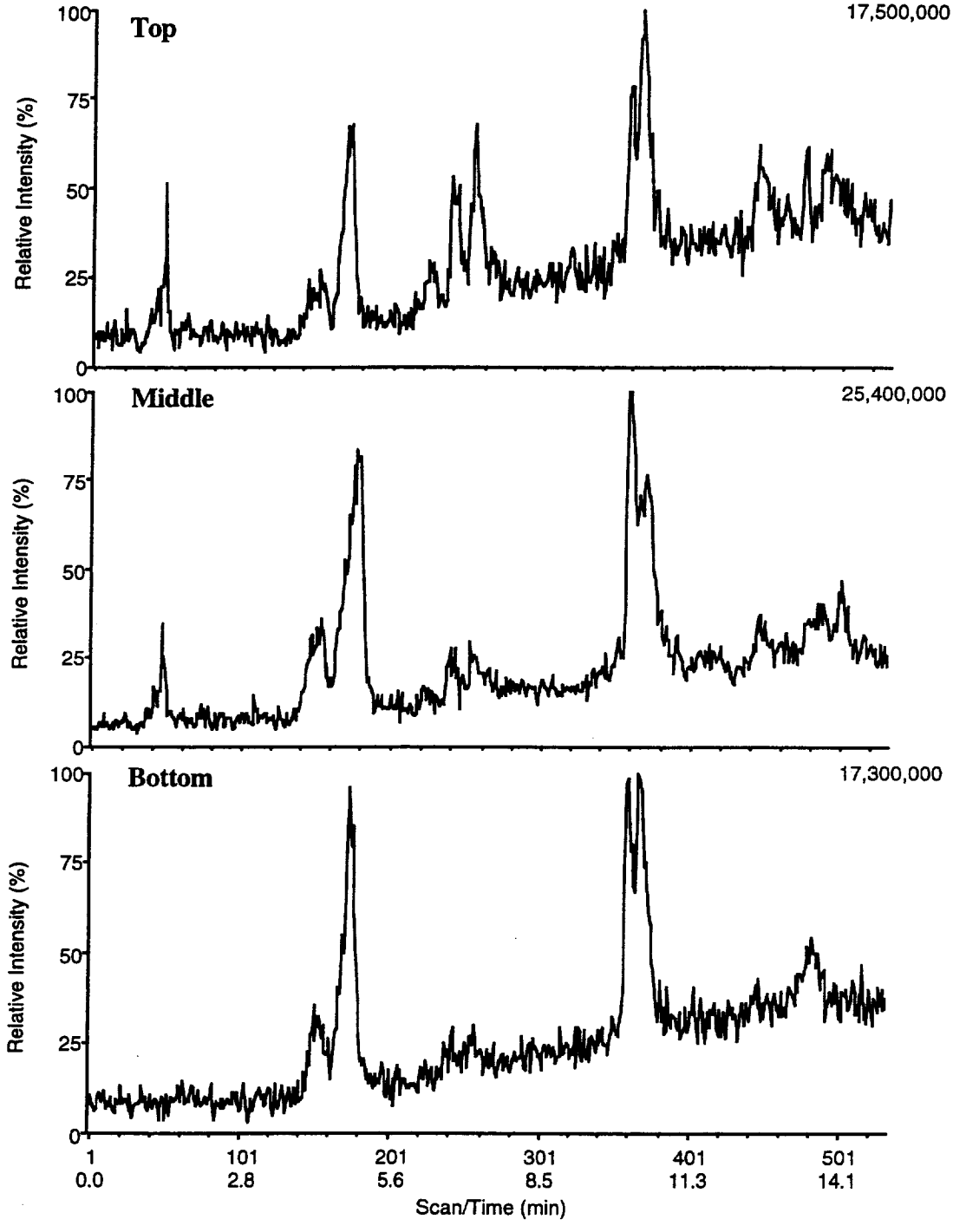

Figure 38. TIC for the Injections of 1 µL of Top, Middle and Bottom Day Seven Solution of Perfluorooctanamide

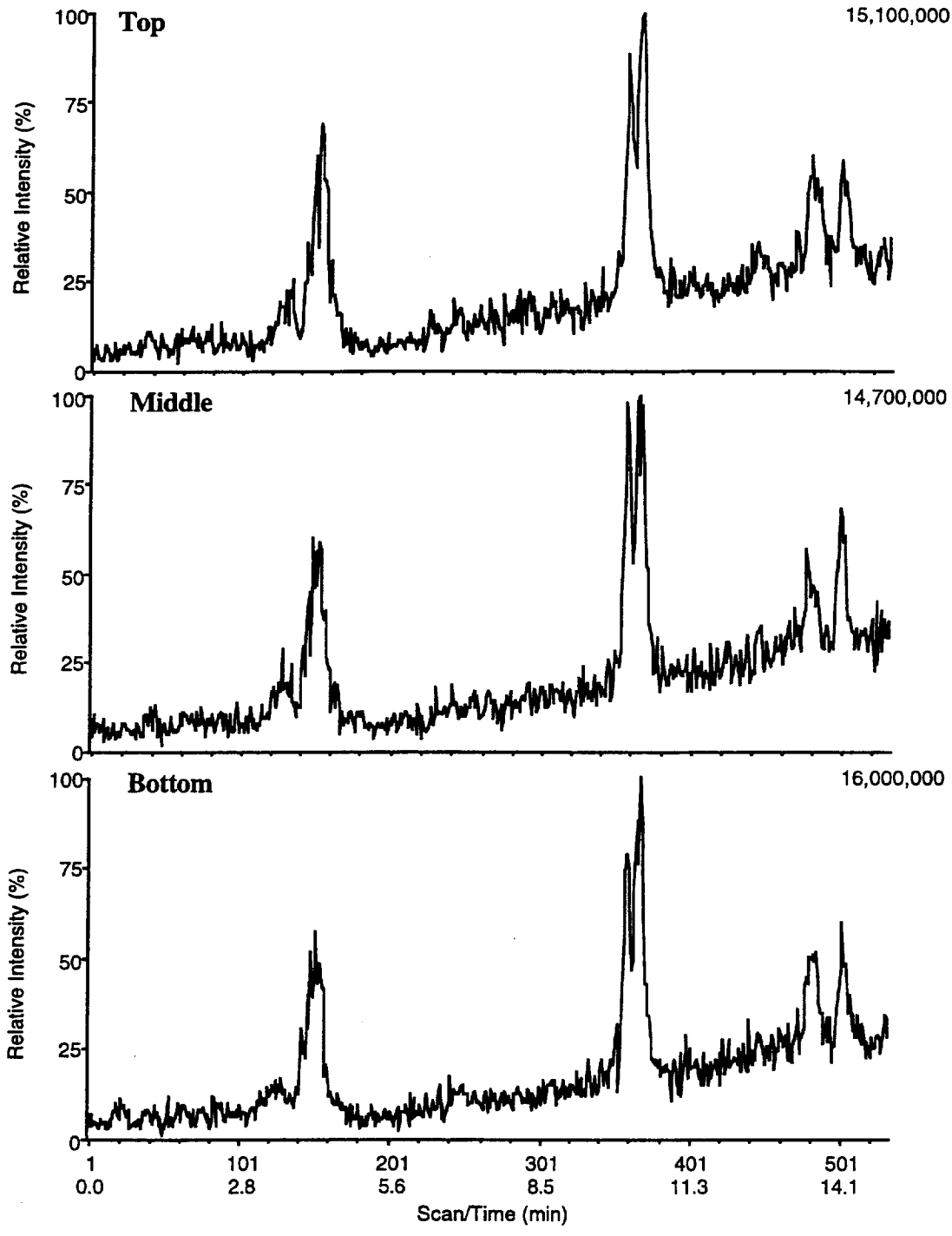

Figure 39. Extracted Ion Chromatograms for the Injection of 1 µL of Day Zero Top Solution of Perfluorooctanamide

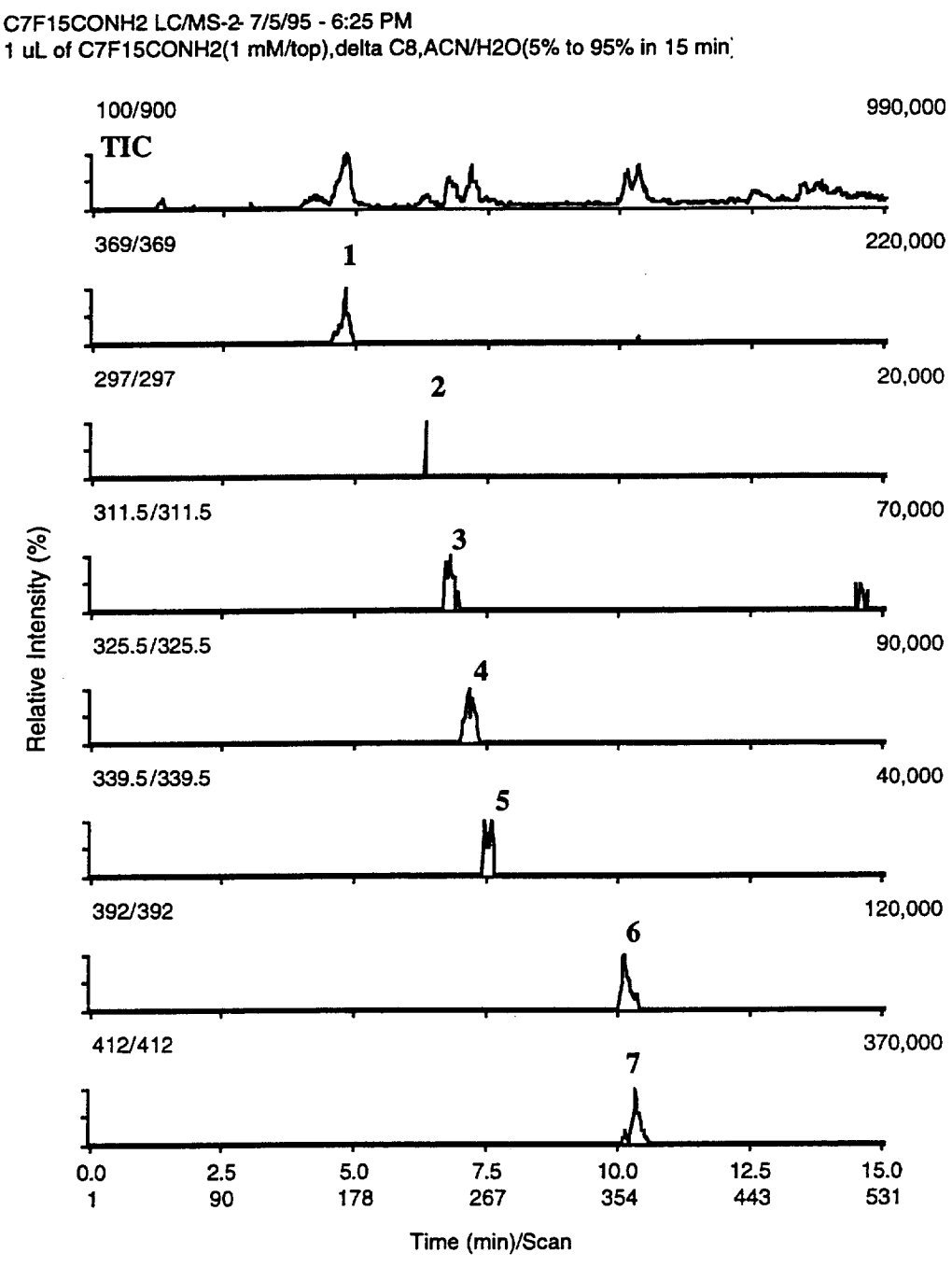

Figure 40. Full-Scan Mass Spectrum of Peak 1 in Figure 39

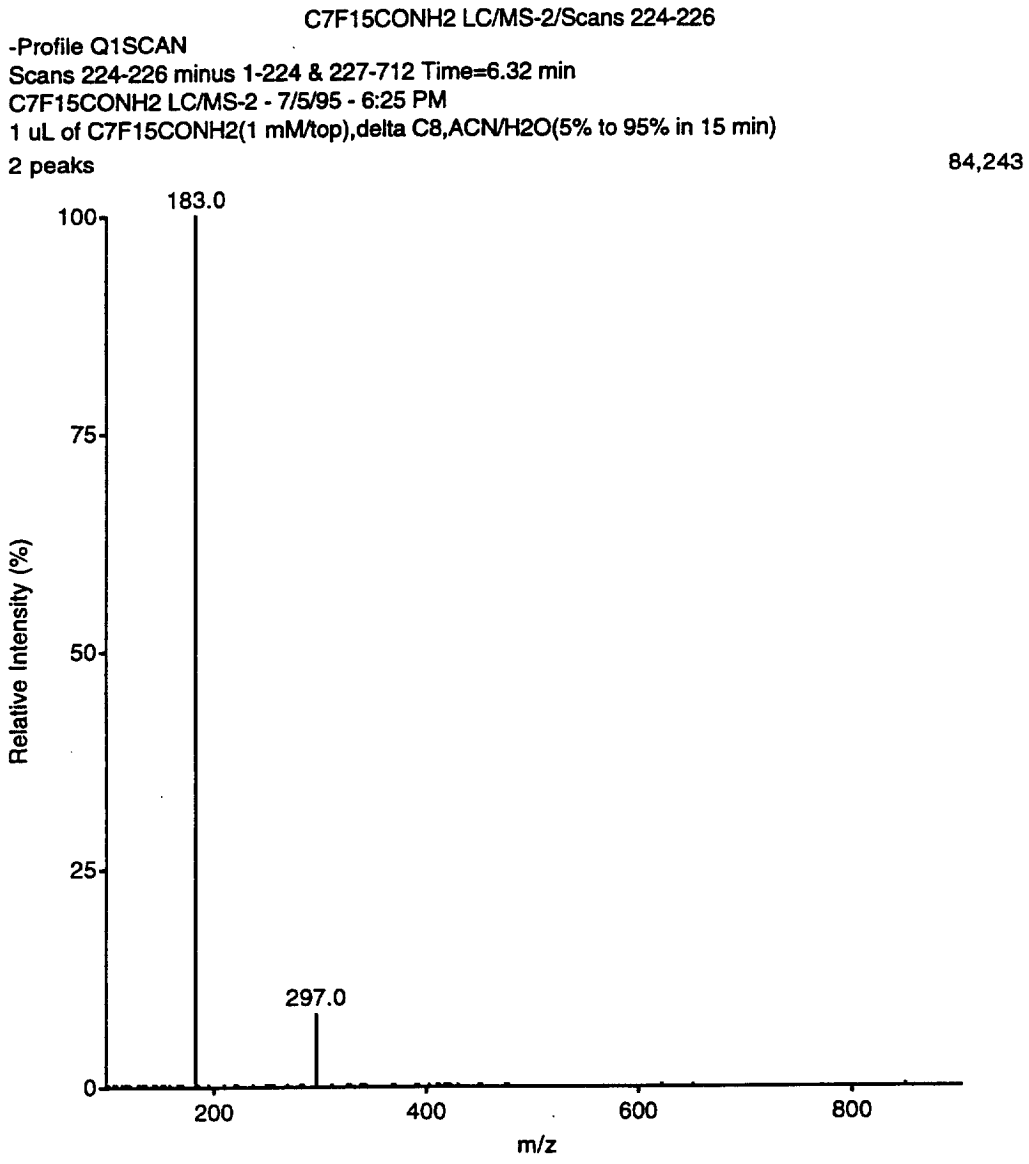

Figure 41. Full-Scan Mass Spectrum of Peak 2 in Figure 39

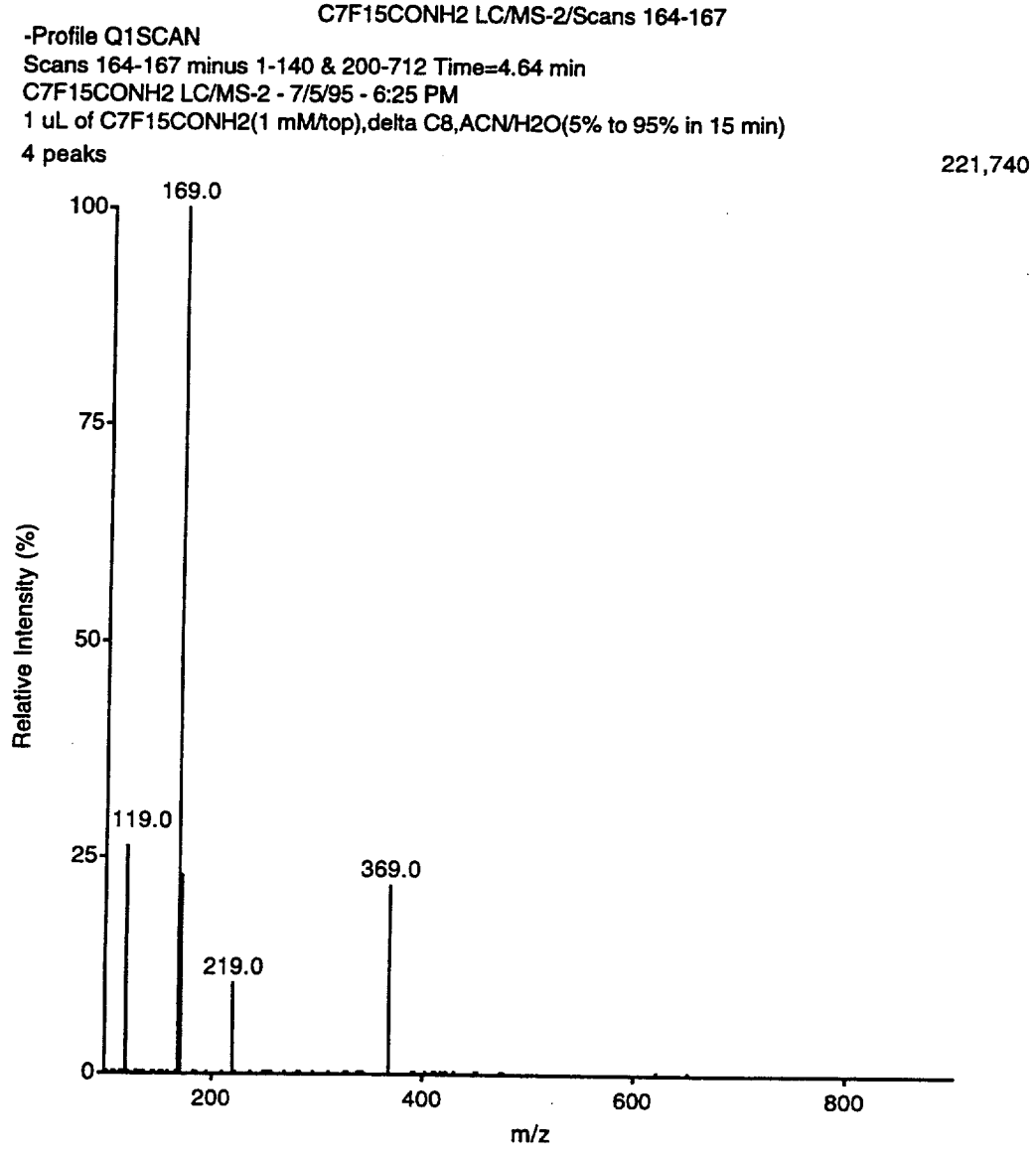

Figure 42. Full-Scan Mass Spectrum of Peak 3 in Figure 39

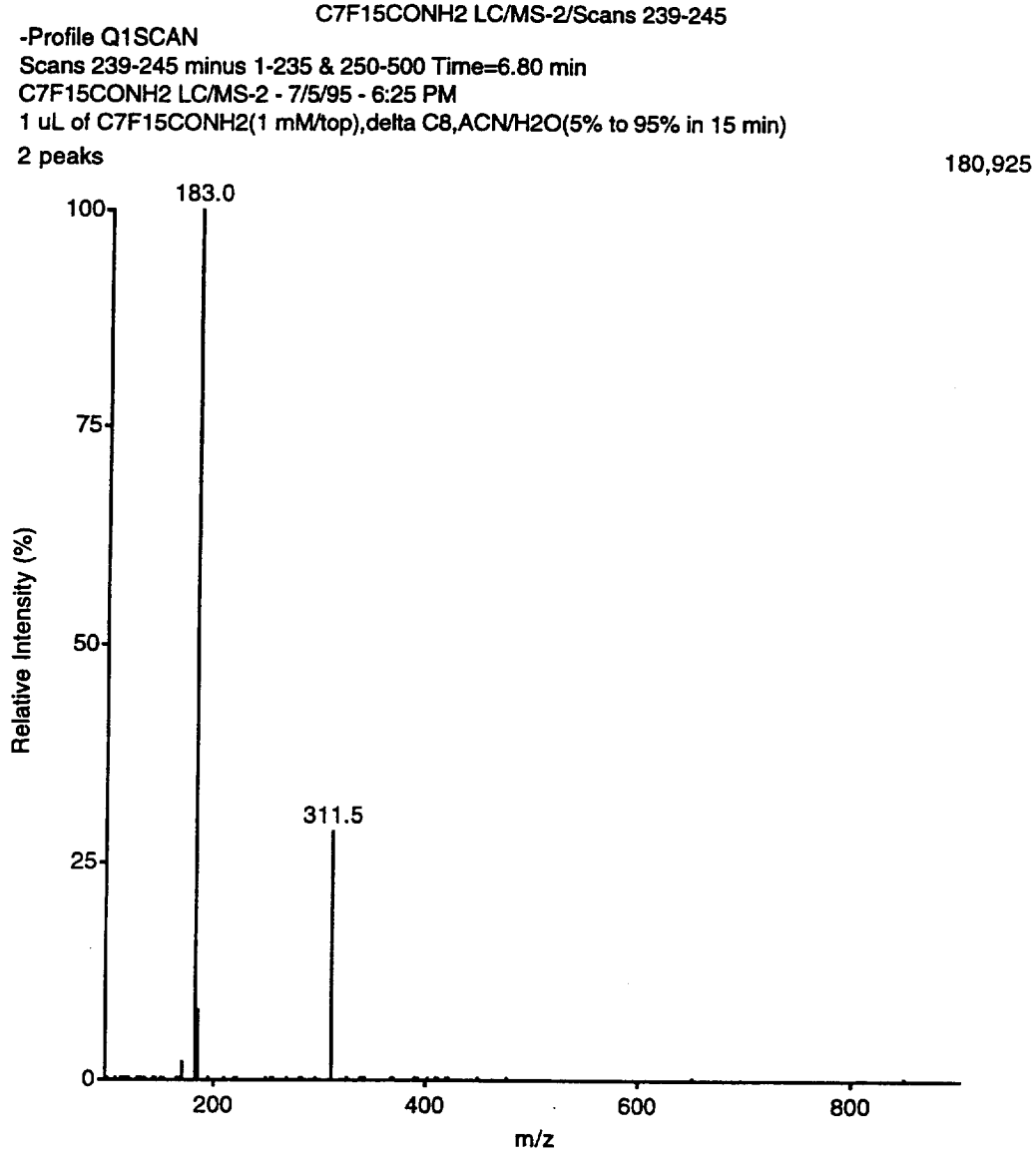

Figure 43. Full-Scan Mass Spectrum of Peak 4 in Figure 39

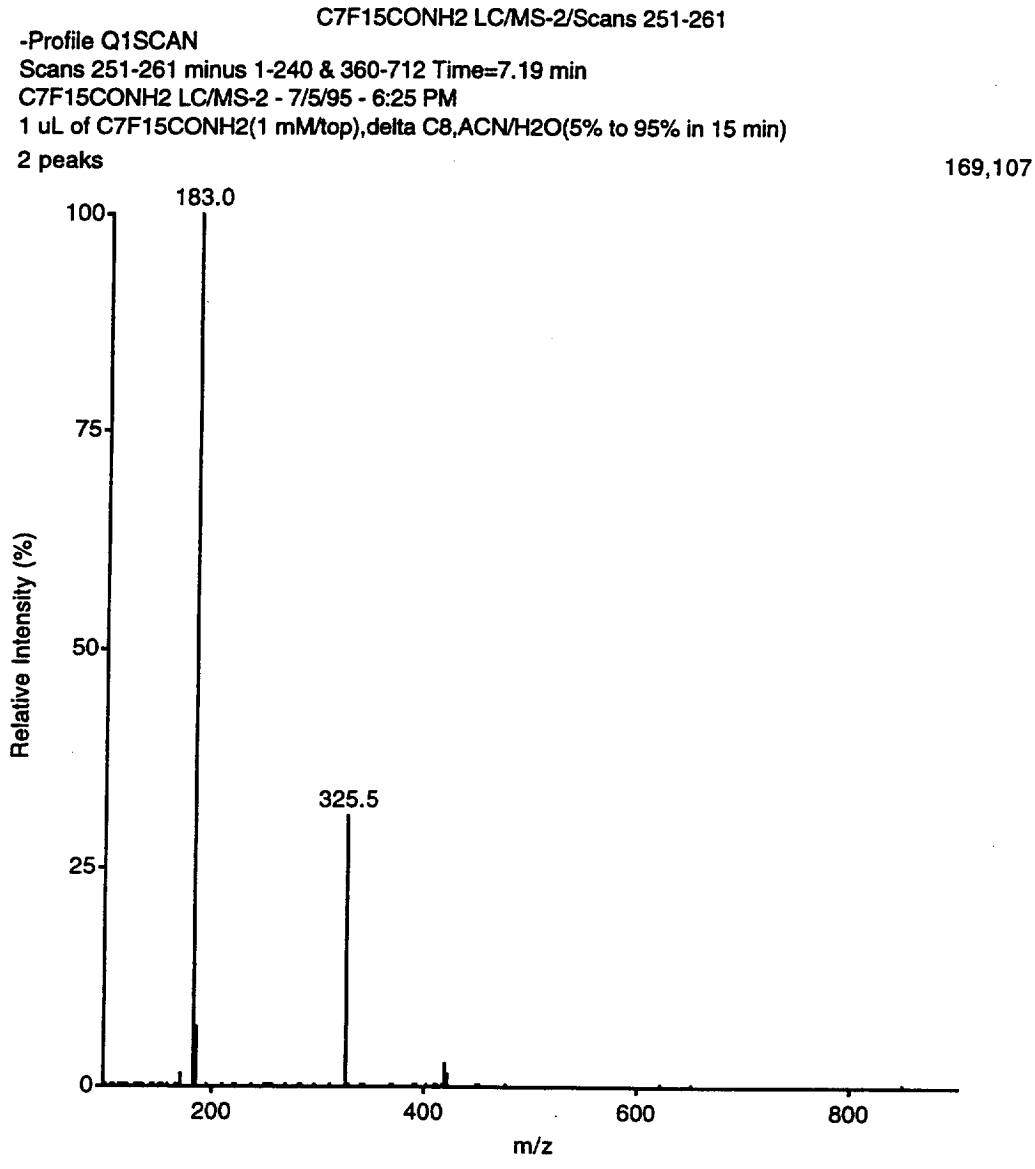

Figure 44. Full-Scan Mass Spectrum of Peak 5 in Figure 39

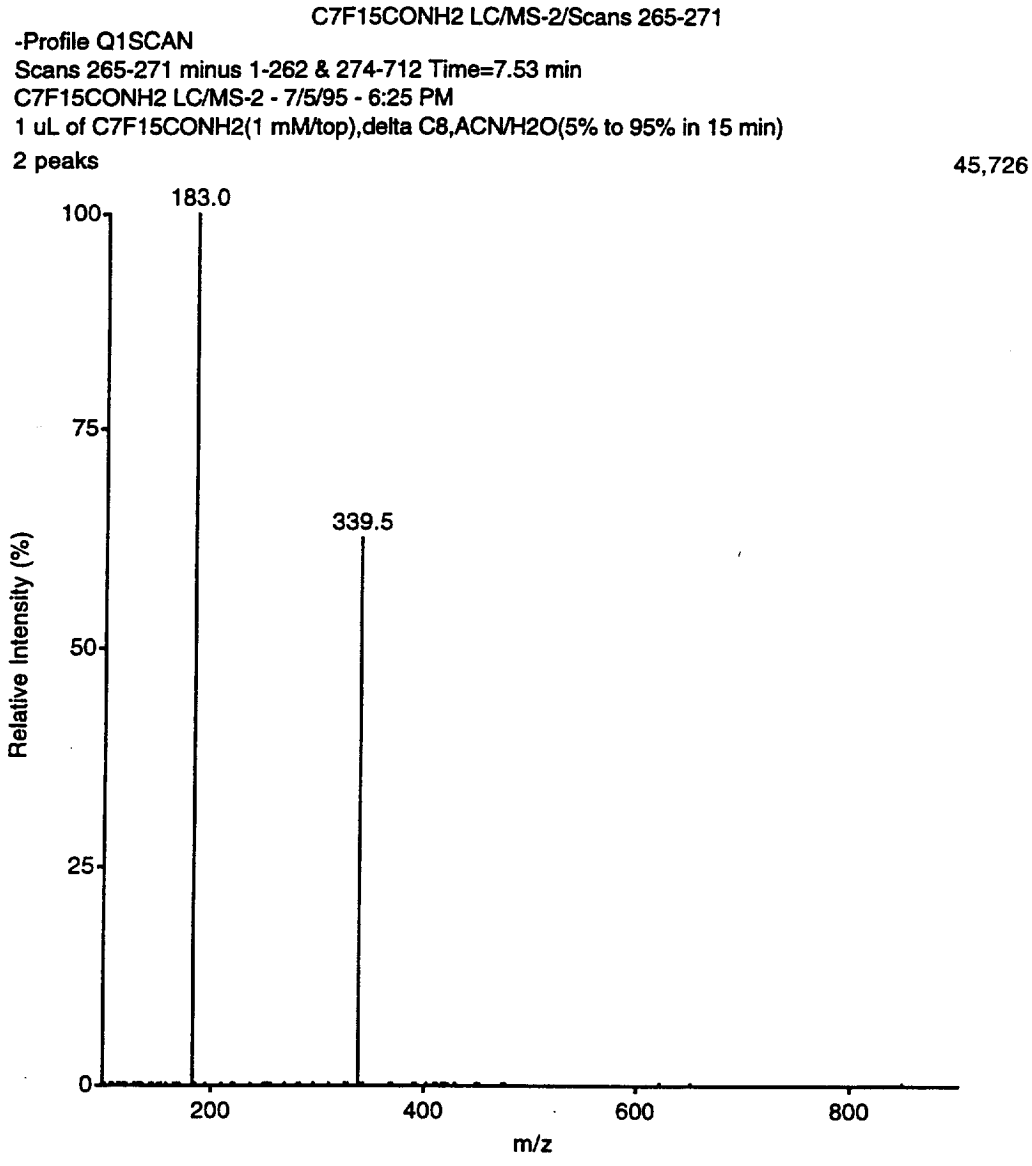

Figure 45. Full-Scan Mass Spectrum of Peak 6 in Figure 39

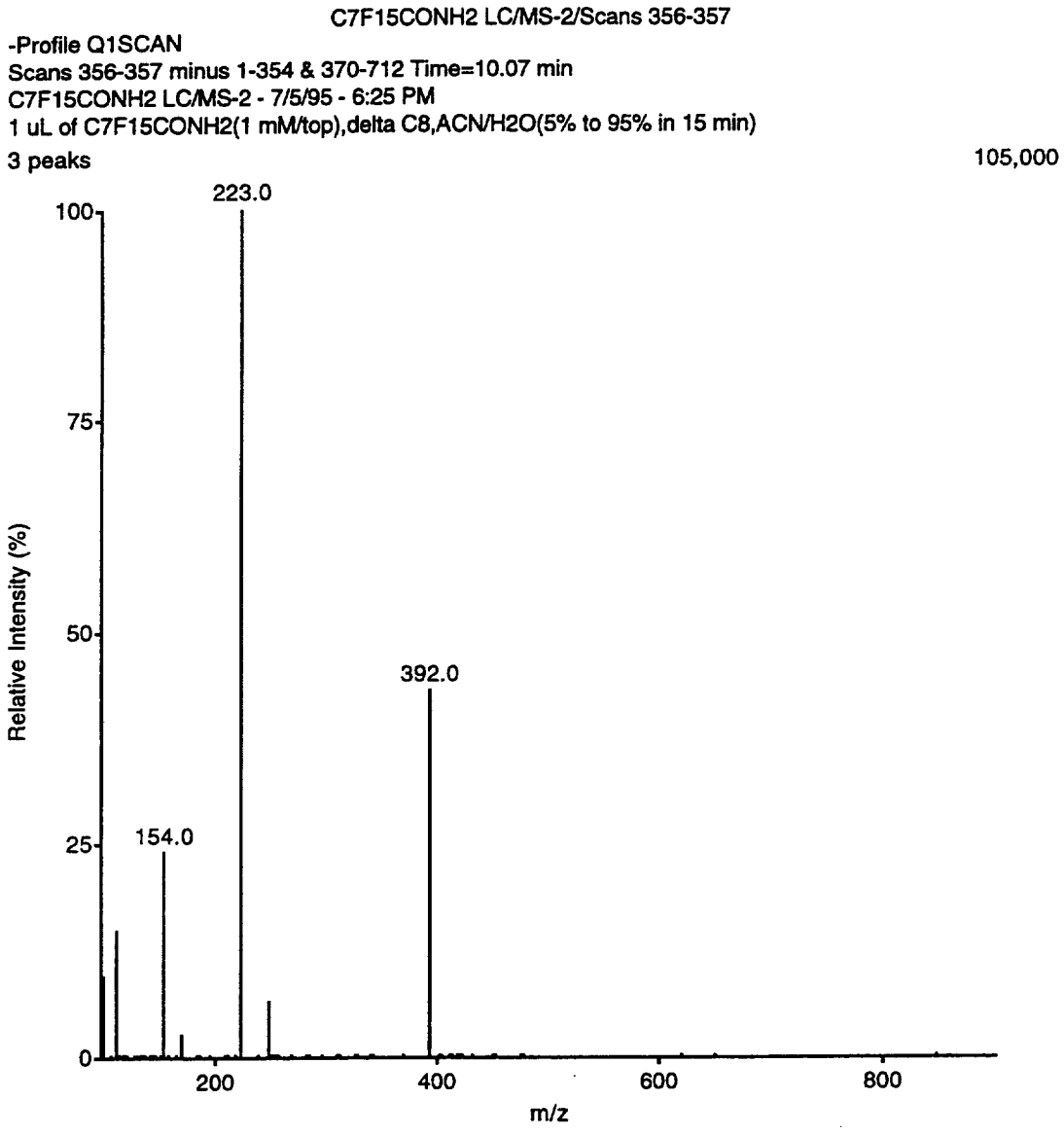

Figure 46. Full-Scan Mass Spectrum of Peak 7 in Figure 39