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**ANALYTICAL REPORT**

**TITLE:** **QUALITATIVE INVESTIGATION OF THE IN VITRO METABOLISM OF T-6292, T-6293, T-6294 AND T-6295 BY RAT AND HUMAN HEPATOCYTES USING ION SPRAY LC/MS AND LC/MS/MS**

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**SIGNATURE PAGE**

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# QUALITATIVE INVESTIGATION OF THE IN VITRO METABOLISM OF T-6292, T-6293, T-6294 AND T-6295 BY RAT AND HUMAN HEPATOCYTES USING ION SPRAY LC/MS AND LC/MS/MS


## ABSTRACT

The *in vitro* metabolism of T-6292, T-6293, T-6294 and T-6295 was investigated using a sensitive and highly specific analytical method developed by Advanced BioAnalytical Services, Inc., Ithaca, NY. This method involves a simple direct injection LC/MS analysis of processed hepatocyte incubate. Siliconized polyethylene transfer pipets and polypropylene autosampler vials were used for all samples. LC/MS and LC/MS/MS experiments were performed in the negative ion mode using an ion spray interface. Chromatography was performed on a 2 mm x 100 mm Betasil ODS column using gradient elution with either aqueous ammonium acetate with methanol or water with methanol. Using a direct injection method, rather than one using extraction, enabled detection of a wide variety of metabolites in a single injection. Included in this report are metabolism data from individual rat and human samples. Several metabolites were found in the rat and human samples and differences were observed between the species.

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# 1. INTRODUCTION

## 1.1 Introduction

The *in vitro* metabolism of T-6292, T-6293, T-6294 and T-6295 in rat and human hepatocyte incubates was investigated using ion spray LC/MS and LC/MS/MS. The quenched incubates were directly injected and separated on an HPLC column. Chromatographic resolution of the various constituents was accomplished using gradient elution with methanol and either water or 2 mM ammonium acetate in water. The mass spectrometer was operated in the full scan (m/z 100 to 800) single MS mode and peaks of interest were then studied by MS/MS techniques for confirmation of selected metabolite peaks.

## 1.2 Study Objective

The objective of this study was to detect and characterize the metabolism of T-6292, T-6293, T-6294 and T-6295 by rat and human hepatocytes.

# 2. EXPERIMENTAL

## 2.1 Chemicals and Materials

T-6292, T-6293, T-6294 and T-6295 (Figure 1) were provided by 3M Medical Department, Toxicology Services, St. Paul, MN 55133.

Siliconized polypropylene autosampler vial: cat # 500 102, Sun Brokers, Inc., Wilmington NC 28402. Siliconized by Eagle Picher Industries, Inc., Miami, OK 74354

Siliconized polypropylene screw-cap tubes: 16 X 101 mm, Cat # 60.541, Sarstedt Inc., Newton, NC 28658, siliconized in-house.

## 2.2 LC/MS Instrumentation

Liquid chromatography was performed using either two Shimadzu LC-10AD pumps (Shimadzu Scientific Instruments, Inc., Columbia, MD 21046) with a Waters WISP 717 plus autosampler (Waters, Milford MA 01757) or a Hewlett-Packard 1090L liquid chromatograph (Hewlett-Packard, Avondale, PA 19311). A Betasil C18 column, 2 mm x 100 mm, was obtained from Keystone Scientific,

Inc., Bellefonte, PA 16823. The mass spectrometers used were an API III$^+$ and API 300 from PE-SCIEX, Concord, Ontario.

## 2.3 Standard Solutions

Since certificates of analysis were not available for the test compounds, 100% purity was assumed, although in the case of T-6293, the purity was known to differ significantly from this value. Stock solutions of T-6292, T-6293, T-6294 and T-6295 were prepared at a concentration of 10 mM in dimethyl sulfoxide. Working solutions (10 µg/mL) of each standard were prepared in 1:1 acetonitrile:2 mM ammonium acetate. All stock solutions and working solutions were stored at 4°C and were allowed to equilibrate to room temperature before use.

**Preparation of Analytical Standard Stock Solutions**

**Stock Solutions of T-6292, T-6293, T-6294 and T-6295 (10 mM):** Accurately weigh each test article on a micro balance and transfer it to a 16 X 101 mm siliconized polypropylene tube. Dissolve the solid sample in an appropriate amount of DMSO to yield a 10 mM solution.

**Working Solutions of T-6292, T-6293, T-6294 and T-6295 (10 µg/mL):** Dilute an appropriate aliquot of 10 mM stock solution to 10 mL with 1:1 2 mM ammonium acetate:acetonitrile in a 16 X 101 mm siliconized polypropylene tube.

## 2.4 HPLC Eluent Preparation

2 mM Ammonium acetate: Ammonium acetate (154.2 mg) was weighed and transferred into a 1 liter graduated cylinder. After adding water to the 1-L mark, the stirred solution was filtered under vacuum through a 0.45 µm filter.

Methanol: Methanol was filtered under vacuum through a 0.45 µm filter.

## 2.5 Sample Preparation

The metabolism study was carried out at SRI International, Menlo Park, CA. The incubates were quenched with an equal volume of ice-cold methanol, centrifuged and shipped packed in dry ice to Advanced BioAnalytical Services (ABS). The samples were stored at ABS at -20°C and were removed only for transfer of

supernatant to an autosampler vial. After sampling, the autosampler vials were maintained in an ice-water bath at all times except during injection.

## 2.6 Sample Analysis

The samples analyzed included Rat 1 (all test articles, 0 and 6-Hr and control sample 13 (no test article)) and Human #H-116 (all test articles, 0 and 6-Hr). These were extensively studied under a variety of chromatographic and mass spectrometric conditions.

HPLC Conditions: Initial HPLC analyses were successfully performed using gradient elution with water and methanol only. Later in the study, it was found that ammonium acetate was required for elution of T-6293. Thus, water was replaced by 2 mM ammonium acetate for further analytical work. Because two different gradient pumps were used for this work, it was difficult to reproduce the gradient obtained using the HP-1090L with the Shimadzu LC-10AD, thus for consistency within gradient systems, the same gradient program was used for both systems. Although this resulted in some variability in retention times between days, depending on the system used, all comparable samples were analyzed with the same system.

Gradient Program:

| Time (Min) | %Methanol | %Water (2mM Amm.Acetate) |
|---|---|---|
| 0 | 20 | 80 |
| 5 | 95 | 5 |
| 9 | 95 | 5 |
| 10 | 20 | 80 |
| 15 | 20 | 80 |

Mass Spectrometer Conditions:

The mass spectrometer was operated in the ion spray mode with negative ion detection. The curtain gas was ultra-high purity nitrogen. Although MS and MS/MS conditions were varied for different analytes, the main differences were in ion spray voltage, orifice voltage and collision energy. No positive ionization work was performed, as the analytes of interest did not ionize under these conditions. It

is conceivable that additional metabolites could be detected using positive ionization, and this should be a part of further experimentation.

The approximate MS and MS/MS acquisition parameters used for the API III$^+$ are listed below. Similar parameters were used for the work performed on the API 300.

| | **MS Mode** | **MS/MS Mode** |
|---|---|---|
| Ion Spray Voltage | -3600 V | -3600 V |
| Orifice Voltage | -70 V | -70 V |
| Declustering Potential | 42V | 42V |
| Curtain Gas (U.H.P. $N_2$) | 1.2 LPM | 1.2 LPM |
| Nebulizer Gas ($N_2$) | 60 PSI | 60 PSI |
| Collision Energy | | 18 eV |
| CGT | | 250 |
| Scan Range | 100-800 amu | variable |
| Step Size | see Note below | variable |
| Dwell Time | 3 msec | 4 msec |

Note: Initial LC/MS step sizes of 1 amu were used to locate the chromatographic peaks of potential metabolites. Once located, step sizes of 0.1 amu were used for mass confirmation.

Instrument mass axis calibration was performed by infusion of PPG-425 calibration solution (polypropylene glycols, average molecular weight 425, dissolved in 1:1 methanol:2 mM ammonium acetate containing 0.1% formic acid and 0.1% acetonitrile) at a flow rate of 10 µL/min in the positive ion mode of detection. Peak widths were approximately 0.6 amu at half-height in the single MS mode. A calibration check was performed for the analytes on Q1 and Q3 by infusion of a 1 µg/mL solution in 1:1 acetonitrile:2 mM ammonium acetate at a flow rate of 10 µL/min. The mass spectrometric parameters and sensitivity were optimized using this solution at 50 µL/min.

## 2.7 Data Handling

All raw chromatographic and mass spectrometric data were stored on the ABS file server ABS1_FS.DATA in the file hierarchy "RAWDATA: Development: 3M: 3M

Metabolism". All pertinent experimental information was stored in ABS notebook No. 1067.

## 3. RESULTS AND DISCUSSION

Initial investigations of the *in vitro* metabolism of the test articles were performed using direct injection of the quenched hepatocyte incubates rather than sample extraction. The reason for this is that, due to the polar nature of these compounds and suspected metabolites, it was felt that extraction recoveries would be low and important metabolites could be missed. Most of the mass spectrometric information obtained in this study were from Q1 scanning experiments, followed by product ion scans. Precursor ion scans of logical CID product ions were used but resulted only in confirmation of previously found metabolites present at high levels. Neutral loss experiments were even less sensitive under the conditions used and provided little information.

### 3.1 Mass Spectrometric Analysis Results for Test Articles

Structures of T-6292, T-6293, T-6294 and T-6295 are shown in Figure 1. The negative ionization mass spectra obtained from infusion of the individual analytes are shown in Figure 2. It should be noted that T-6292 could not be ionized in either the negative ionization mode, since it has only a very weakly acidic alkyl hydroxyl group or the positive ionization mode, since the most basic site is a tertiary nitrogen of a sulfonamide.

The fact that T-6293 exists mainly in the diphosphoester form[1] ($M_r$ =1204), comprising 82% of T-6293 solids, accounts for the reduced response seen for the monoester ($M_r$= 651), when compared to T-6294 and T-6295. The diester was not detectable under the conditions used in this study. The negative ionization collision induced dissociation (CID) full-scan spectra of the compounds are presented in Figure 3. T-6295 was found to be extremely stable and required both a high orifice voltage and high collision energy for fragmentation. A summary description of the proposed plausible CID fragmentation pathways are shown in Figures 4-6. Several of these fragments were diagnostic for metabolite identification.

### 3.2 Metabolism by Rat Hepatocytes

Metabolism of the test articles by rat hepatocytes was examined using a Sciex API III$^+$ mass spectrometer.

### 3.2.1 Metabolism of T-6292 by Rat Hepatocytes

Although T-6292 did not ionize under the conditions used, and was thus not detectable in these experiments, several potential metabolites were nonetheless found. When comparing the total ion chromatogram (TIC) obtained from the control rat sample (Figure 7, top; no test article added) with that obtained from the Rat 1, 0-Hr sample (Figure 8, top, reaction quenched at approximately time 0), several large peaks are observed in the latter over the range of 7.2 to 8.5 min which are absent in the control sample. It should be noted that there is a 0.5 min difference between the TICs, due to operator error; the control TIC peaks are 0.5 min earlier. The 0-Hr peaks were found to contain masses in a homologous series which are compound related but which may also be impurities or degradation products. Each of the observed peaks contains masses which differ by 80 and 107 amu. An example is given in Figure 9. It is suspected that the different ions within each chromatographic peak were generated by CID in the sampling orifice region of the mass spectrometer since no collision gas was used. In the example given, the lowest mass ion, m/z 319, is a characteristic mass for perfluoroalkyl compounds, namely, $C_6F_{13}$. The second ion, m/z 426, is 107 amu higher, which has been previously described for these compounds as a loss of $EtNSO_2$ (Figure 5). The ion observed at m/z 506 is 80 amu higher, which correspond to $SO_3$, a common loss under these conditions. Thus, the proposed structures of this and the other analogs are shown in Figure 10a. It may be somewhat surprising that 0-Hr sample would contain metabolites at significant levels; however, for rapid reactions, the sampling and quenching at this time point may be crucial.

Summary extracted ion chromatograms of the fragment ions analogous to m/z 319, differing only by $CF_2$ groups, are shown in Figure 8. As predicted, no corresponding peak appears for m/z 469 ($C_9F_{19}$), since this would exceed the perfluoroalkyl chain length of the parent molecule. Chromatographic peak splitting is evident in the late eluting peaks, probably due to chromatographic resolution of the branched isomers of these compounds. Corresponding spectra for the Rat 1, 6-Hr sample (Figure 11) indicate a decreased response for these compounds, except for the parent $C_8F_{17}$ fragment (m/z 419). The control sample contained no significant levels of these ions (Figure 7).

An additional homologous series of proposed metabolites of the parent compound and impurities has been observed which appear to be glucuronic acid conjugates.

The masses of these compounds match those of the O-glucuronides (Figure 10b) of these compounds. Figure 12 shows the extracted ion chromatograms for the $[M-H]^-$ of the suspected intact glucuronides in the series for Rat 1, 6-Hr. The glucuronides of the C3 analog through the parent compound, the C8 analog, are presented. By comparison, the peaks are greatly diminished in the 0-Hr sample (Figure 13) and absent in the control sample (data not shown). No evidence was found for any of the possible N-glucuronides.

An additional major metabolite was the N-di-dealkylated perfluoroalkyl sulfonamide (Figure 14) with an $[M-H]^-$ of m/z 498. For this compound, a homologous series was also observed, but only for the C7 and C6 homologs (data not shown). A carboxylic acid metabolite was also found (Figure 14) with an $[M-H]^-$ of m/z 584. A homologous series was found for this metabolite which included the C4-C8 homologs (data not shown). Extracted ion chromatograms of the C8 homologs of these metabolites from Rat 1, 0 and 6-Hr are presented in Figure 15. To support the proposed structure of the carboxylic acid metabolite, an MS/MS product ion scan was performed for precursor ion m/z 584 (Figure 16). The fragment ions formed were consistent with the structure. The parent ion is not observed but a conceivable loss of the three-membered lactone $CH_2CO_2$ from the carboxylic acid anion would produce the ion at m/z 526.

### 3.2.2 Metabolism of T-6293 by Rat Hepatocytes

T-6293 also underwent significant metabolism by rat hepatocytes. The TICs obtained from the control sample, Rat 1, 0-Hr and Rat 1, 6-Hr are presented at the top of Figures 17, 18 and 19, respectively. As with T-6292, a 0.5 min difference exists between the TICs, due to operator error; the control TIC peaks are 0.5 min earlier. Several possible metabolites were found in the rat samples. The extracted ion chromatograms for the anions of these metabolites are also shown in Figures 17-19. None of the metabolites found in the 6-Hr sample were found in either the 0-Hr or control samples. The parent anion at m/z 650 was found in both the 0 and 6-Hr samples, although reduced by half in the latter.

Possible structures for m/z 542 and 556 are shown in Figure 20. Very little "up front" CID occurred for these peaks, thus MS/MS experiments would be required to support the proposed structures. The observed retention times are consistent with the proposed structures, with the acid eluting earlier than the alcohol. The

metabolite observed with an $[M-H]^-$ of m/z 584 was shown to be the carboxylic acid previously seen for T-6292 (see Figure 14) by a MS/MS product ion scan (data not shown). The glucuronide proposed for T-6292 was also seen in T-6293 (Figure 19, m/z 746).

The ions 498 and 499 were present at significant levels in both 0 and 6-Hr samples, although they both had higher responses in the 6-Hr sample (Figure 21). The likely structures of these compounds are perfluorooctanesulfonate (m/z 499, T-6295) and perfluorooctanesulfonamide (m/z 498). From Figure 21, it can be seen that for both 0 and 6-Hr samples, the later m/z 499 peak coincides with the 498 ion. This is believed to be an artifact due to the isotopic contribution of the m/z 498 ion and incomplete mass resolution between m/z 498 and m/z 499. Additionally, the observed retention time for the first peak in the m/z 499 ion chromatogram is closer to that routinely observed for perfluorooctanesulfonate than for the second (see Figure 28). Thus, it is assumed that the second m/z 499 ion is actually m/z 498 being detected as m/z 499. The m/z 499 ion was found at significantly high levels in all control samples examined (data not shown) at the same retention time as in these samples. Thus, while it may also be a product of metabolism, there is an endogenous background level as well.

Two additional ions were found in the TIC, m/z 327 and m/z 419 (Figure 22). The ion at m/z 419 is a common fragment ion (see Figure 4) and an easily conceivable product of degradation and/or metabolism while m/z 327 is more difficult to rationalize and may be a product of "up front" CID with a parent ion higher than the scan range used. Further experimentation would be required. In the control sample, neither ion was detected (not shown).

### 3.2.3 Metabolism of T-6294 by Rat Hepatocytes

The TICs and extracted ion chromatograms for the proposed metabolites of T-6294 from the control, 0-Hr and 6-Hr samples for Rat 1 are presented in Figures 23-25, respectively. The parent compound ($[M-H]^- = 526$) was observed in both 0 and 6-Hr samples at approximately 8.7 min. Several metabolites were found in the rat hepatocyte samples for T-6294. The main metabolite was perflurorooctane sulfonamide ($[M-H]^- = 498$, 8.0 min), the N-dealkylation product. This metabolite was observed in both the 0 and 6-Hr samples, although it was much larger in the latter. A MS/MS product ion scan was performed on the 6-Hr sample for precursor

ion m/z 498 (Figure 26), and all observed fragments supported the proposed structure. The structures for the radical species shown in Figure 26 are not proposed to be the most stable configurations. The metabolite observed at m/z 483 (6.6 min) is postulated to be the same as the fragment ion proposed in Figure 16. The remaining metabolites at m/z 542 and 556 are postulated to be those in Figure 20, with oxidation occurring on the terminal methyl group.

### 3.2.4 Metabolism of T-6295 by Rat Hepatocytes

The control blank, 0-Hr and 6-Hr TICs for T-6295 in Rat 1 are presented in Figures 27-29, respectively. As previously stated (Section 3.2.2), the 499 ion was observed observed in all control samples. The remaining ions in the extracted ion chromatograms are members of the homologous series seen previously for T-6292. It is difficult to believe that these are products of metabolism since their formation would require deletion of $CF_2$ units from the chain. Apparently, T-6295 is metabolized minimally by rat hepatocyte incubates, as judged by the high levels of parent in both the 0 and 6-Hr samples.

## 3.3 Metabolism by Human Hepatocytes

Metabolism of the test articles by human hepatocytes was similar to that of the rat but fewer types were seen. The human samples were examined using a Sciex API 300 mass spectrometer, however, the investigation was less extensive than that of the rat samples. Further work is certainly warranted. Since no new metabolism was discovered for the human samples, the results will only be discussed using text; no figures are presented.

### 3.3.1 Metabolism of T-6292 by Human Hepatocytes

The homologous series presented in Figure 10a was not observed in the human. Interestingly, however, the glucuronide series presented in Figure 10b was seen in the 6-Hr samples. The sulfonamide in Figure 14 was observed at very low levels and the acid in Figure 14 was not detected at all.

### 3.3.2 Metabolism of T-6293 by Human Hepatocytes

The parent ion was the only compound-related moiety seen in these samples; however, with optimization of the mass spectrometer focused on T-6293, further information should be attainable. Based solely on abundance the parent ion is one-

third lower in the 6-Hr sample than in the 0-Hr sample, thus some metabolism may have occurred.

### 3.3.3 Metabolism of T-6294 by Human Hepatocytes

The parent and sulfonamide metabolite (m/z 498, see Figure 14) were the only compound-related ions observed in the samples. The abundance of the parent ion in the 0-Hr sample was reduced approximately by half in the 6-Hr sample and, conversely, the abundance of the sulfonamide in the 0-Hr sample was approximately doubled in the 6-Hr sample.

### 3.3.4 Metabolism of T-6295 by Human Hepatocytes

There were no discernable differences between the 0 and 6-Hr sample for T-6295.

## 4. CONCLUSIONS

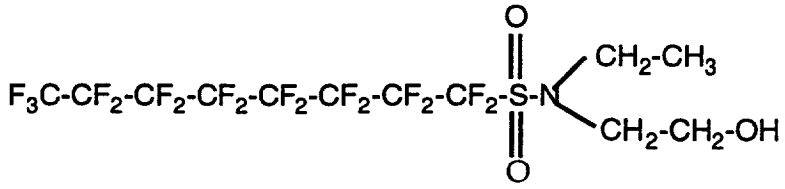
Several potential metabolites and impurities were determined from the LC/MS data in the study. Further MS/MS experimentation, possibly in conjunction with a sample cleanup and concentration step, would be required to further support the postulated structures in the rat samples. Additionally, for the human samples, further high sensitivity detection experimentation is required for complete preliminary investigation, followed by MS/MS experimentation for confirmation of the metabolite structures.

## 5. REFERENCES

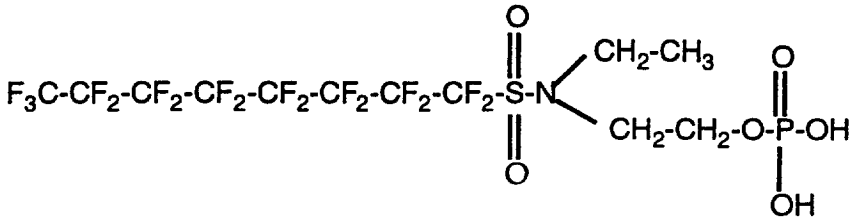
1. Gordon, Steven C., Ph.D., D.A.B.T., Health Hazard Summary of Ammonium Salts of Mono-, Di- and Tri[N-ethyl(perfluorooctane)-sulfonamidoethyl}phosphate (FC-807). September 14, 1994. Supplied by 3M Company.

**Figure 1. Structures of the study test articles**

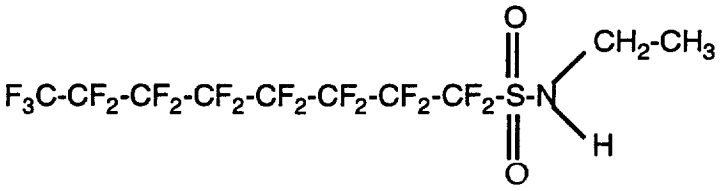
T-6292
$M_r = 571$




T-6293
$M_r = 651$




T-6294
$M_r = 527$




T-6295
$M_r = 500$

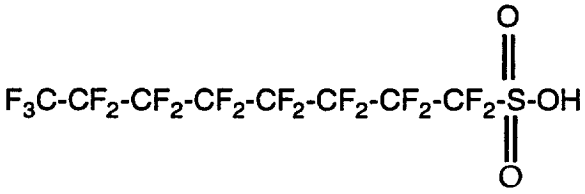


**Figure 2.** **Negative ionization mass spectra obtained from the test articles T-6293 ([M-H]$^-$ = 650.1), T-6294 ([M-H]$^-$ = 526.1) and T-6295 ([M-H]$^-$ = 498.9)**

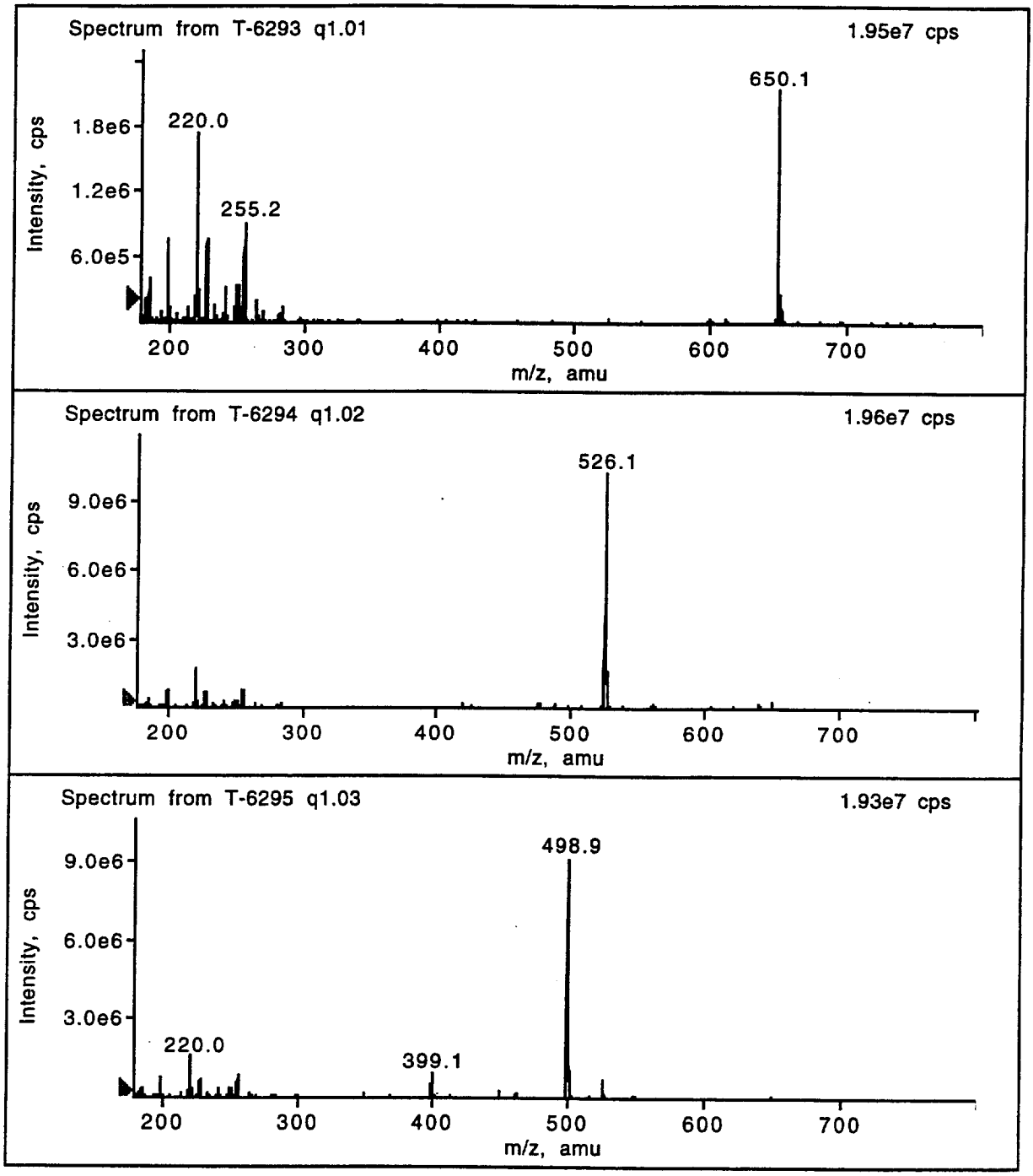


**Figure 3. Product ion spectra obtained from the deprotonated test articles T-6293, T-6294, and T-6295.**

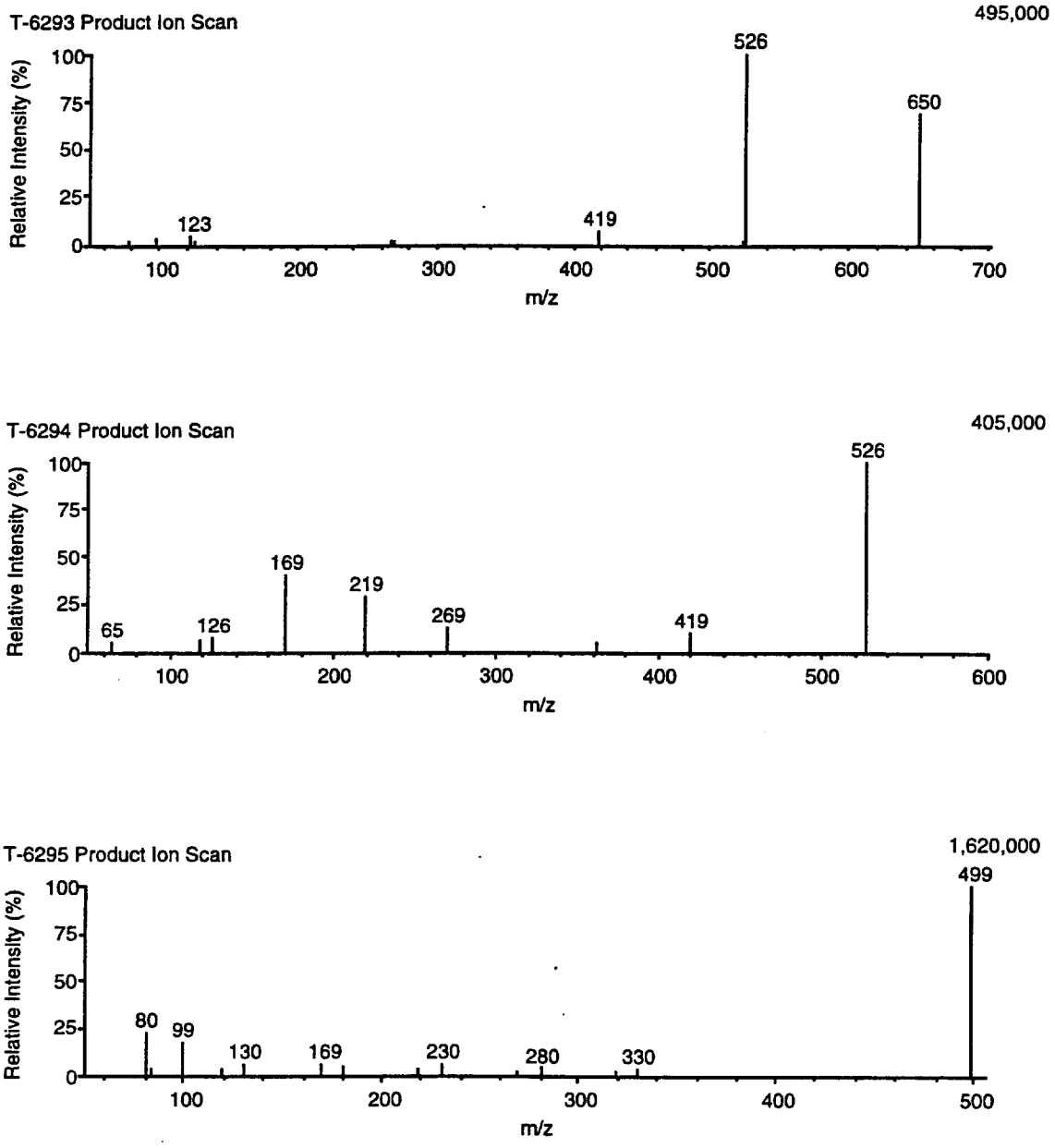


**Figure 4. Proposed CID fragmentation mechanism for T-6293.**

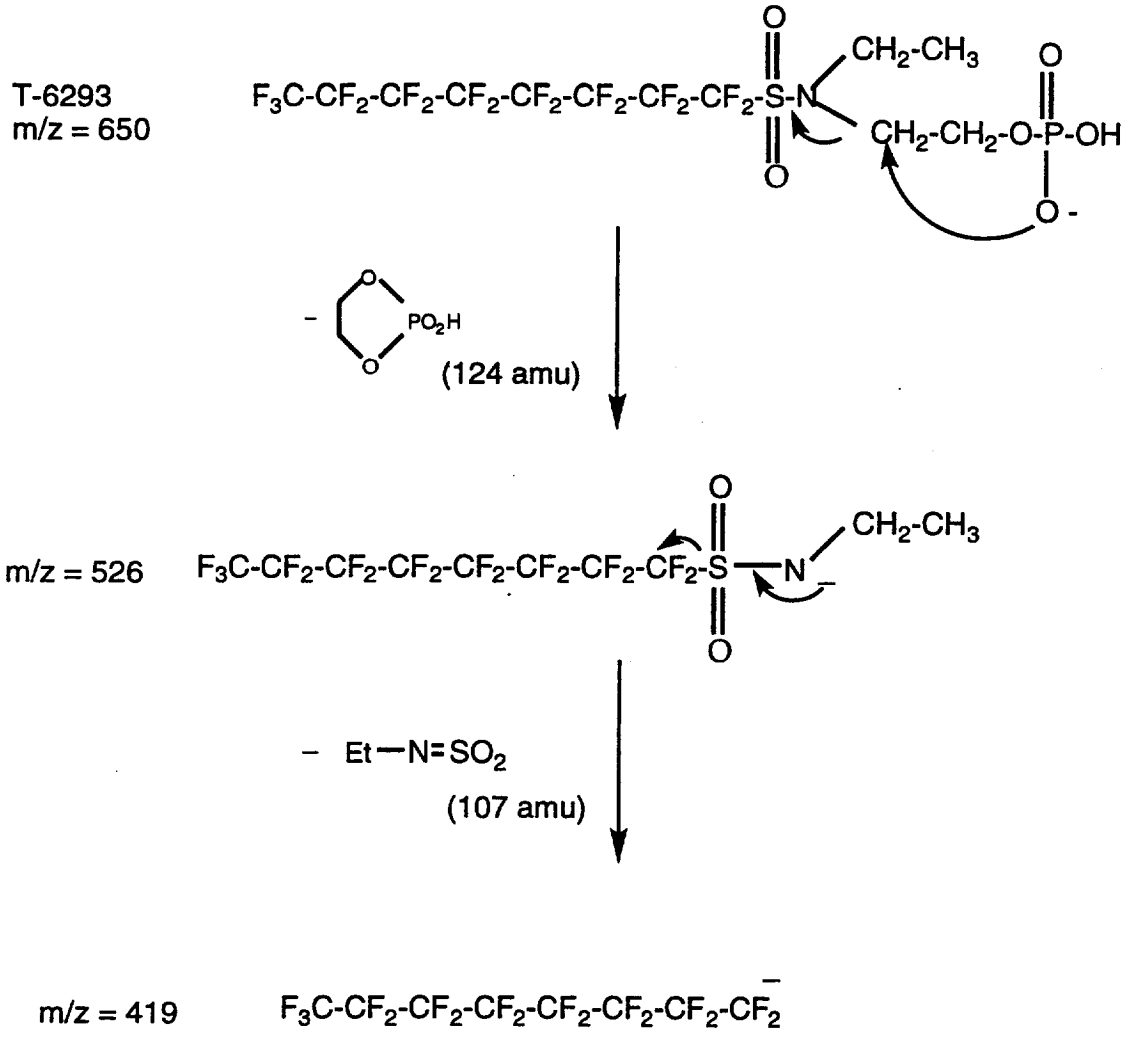


**Figure 5. Proposed CID fragmentation mechanisms for T-6294.**

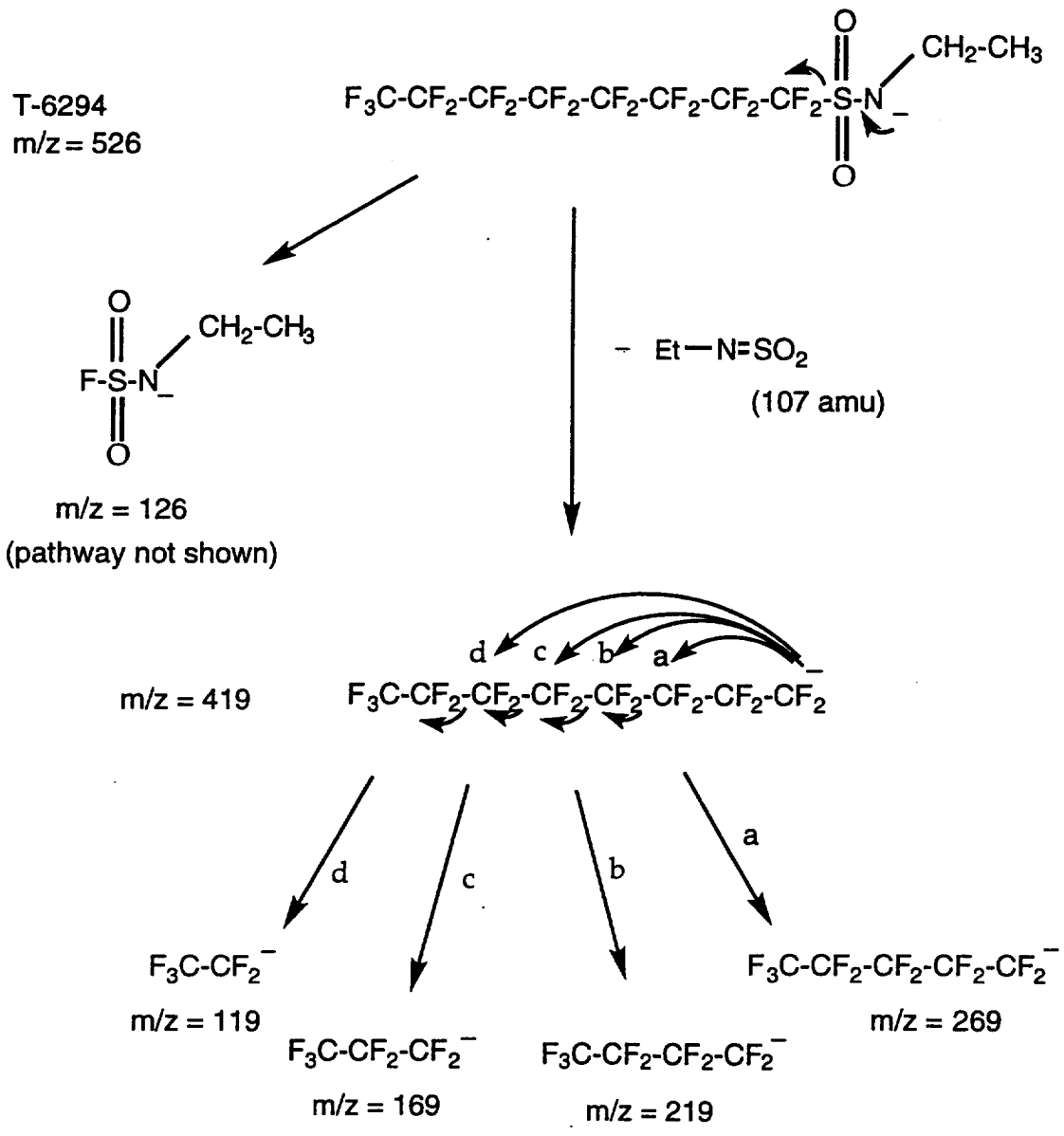


**Figure 6. Proposed CID fragmentation mechanisms for T-6295.**

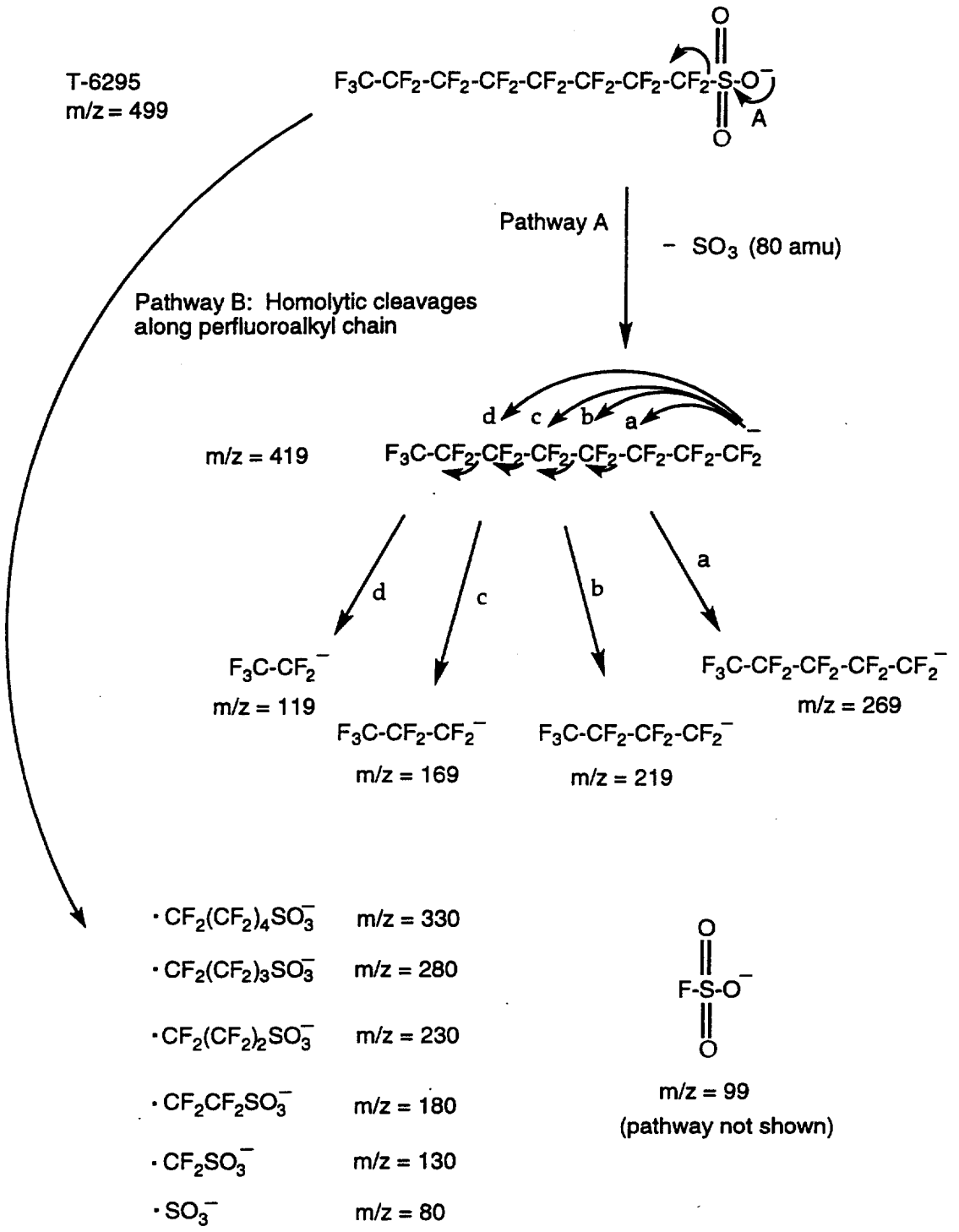


**Figure 7. Total ion and extracted fragment ion chromatograms for T-6292 obtained from Rat Control Sample 13.**

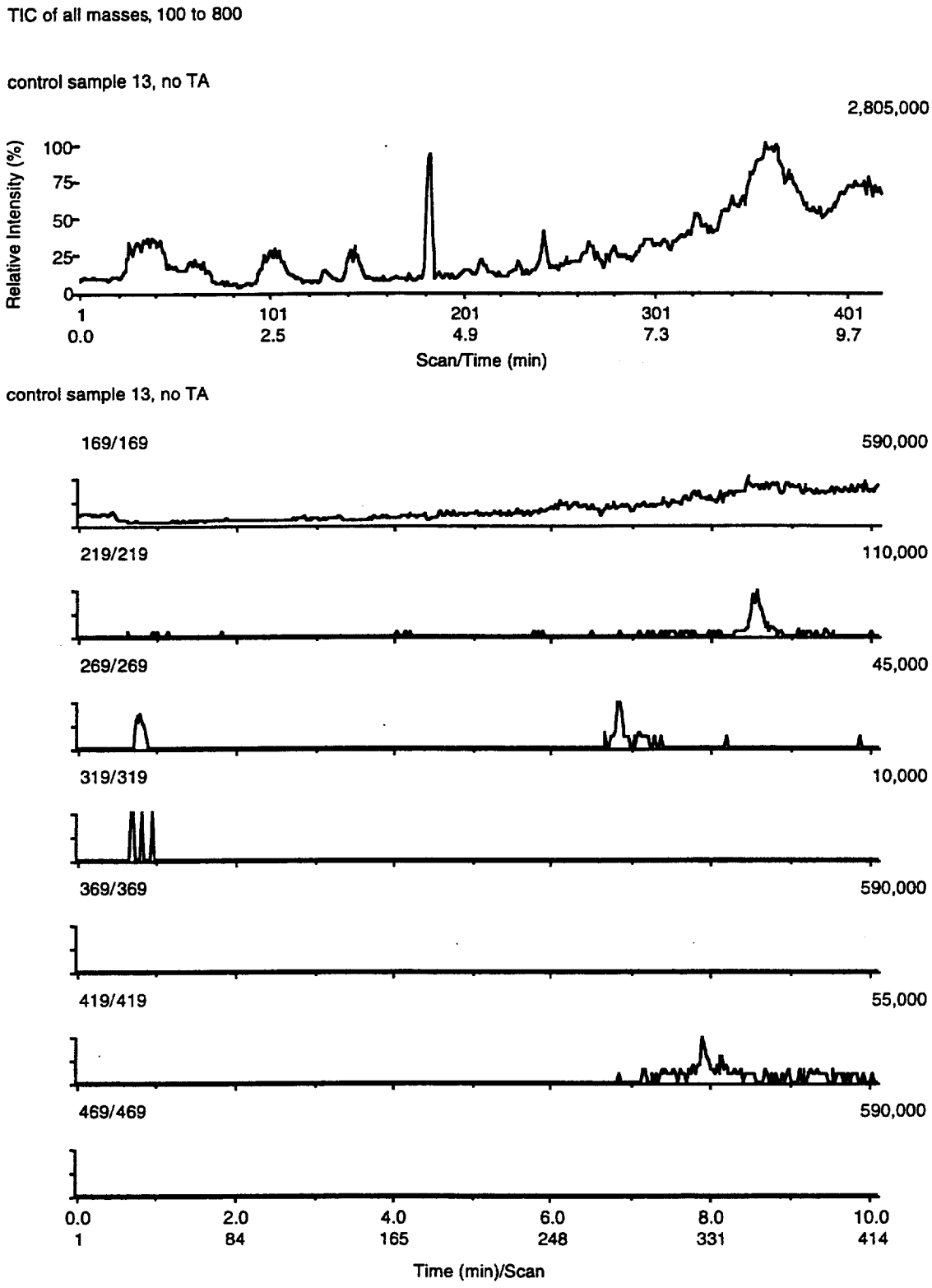


**Figure 8. Total ion and extracted fragment ion chromatograms for T-6292 obtained from Rat 1, 0-Hr sample.**

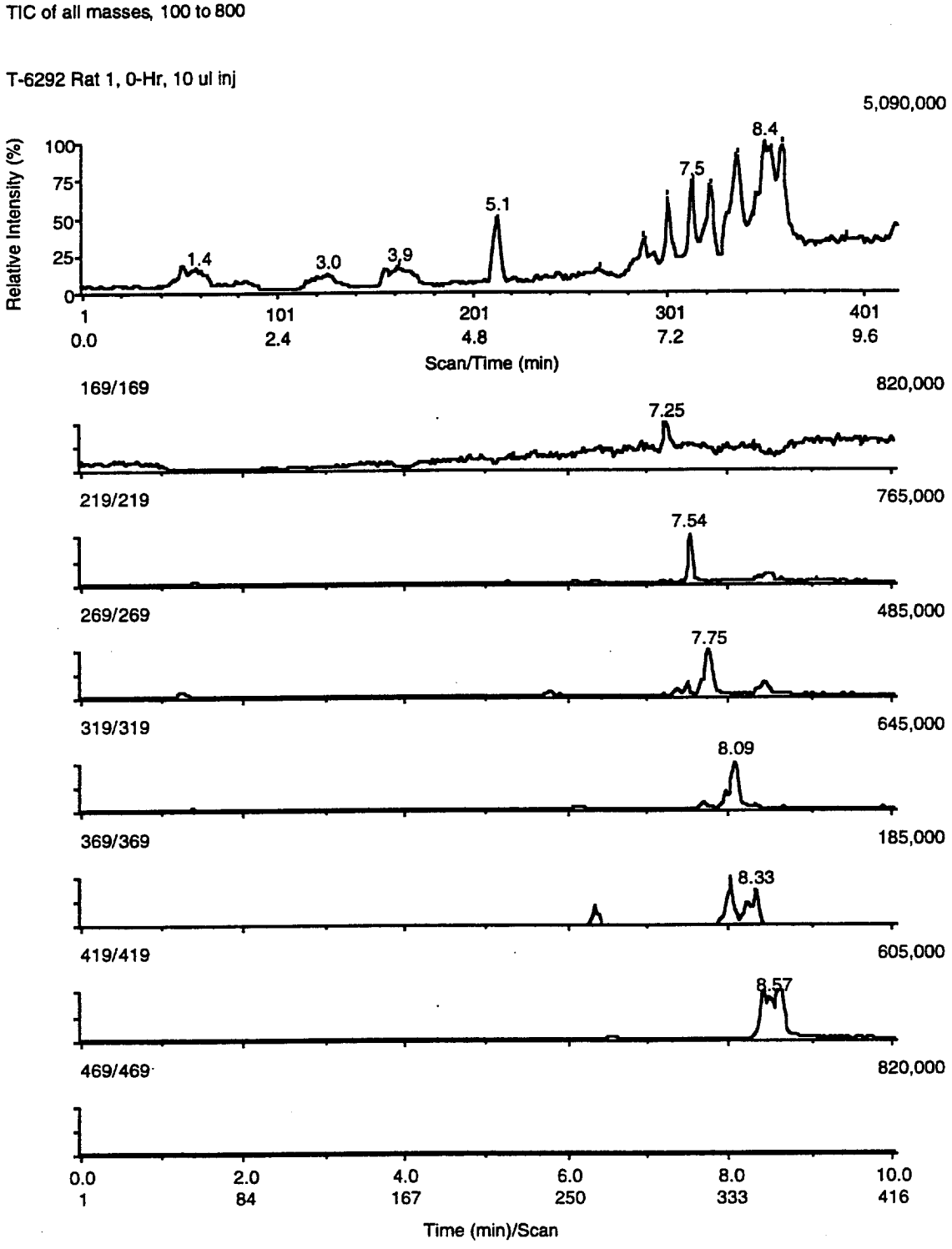


**Figure 9. Extracted ion chromatograms of fragment peaks for T-6292 homologous series in Rat 1, 0-Hr sample.**

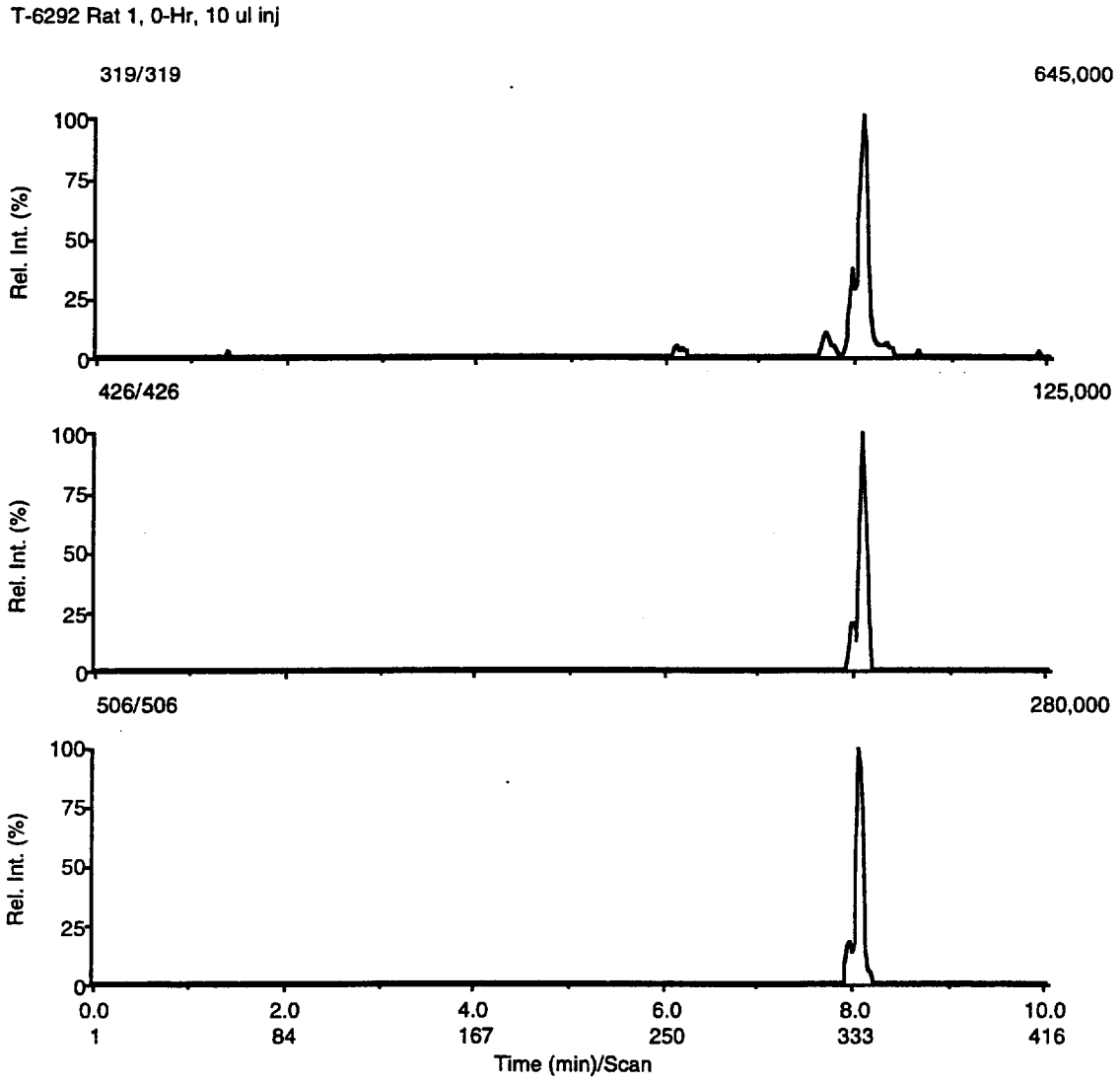
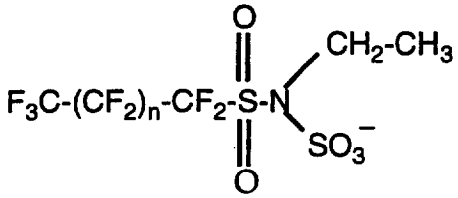
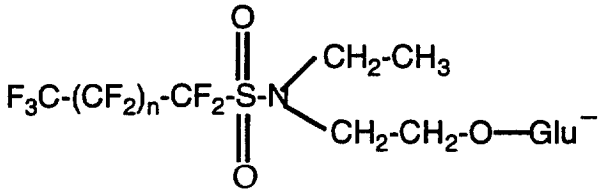


**Figure 10a. Proposed structure of parent compound for T-6292 homologous series from Figures 8 and 11.**




| Mass of Parent Anion | n | Corresponding Fragment Ion in Figures 8 and 11 |
|---|---|---|
| 356 | 1 | 169 |
| 406 | 2 | 219 |
| 456 | 3 | 269 |
| 506 | 4 | 319 |
| 556 | 5 | 369 |
| 606 | 6 | 419 |

**Figure 10b. Proposed structure of glucuronide for homologous series from Figures 12 and 13.**




| Mass of Parent Anion | n |
|---|---|
| 496 | 1 |
| 546 | 2 |
| 596 | 3 |
| 646 | 4 |
| 696 | 5 |
| 746 | 6 |

**Figure 11. Total ion and extracted fragment ion chromatograms for T-6292 obtained from Rat 1, 6-Hr sample.**

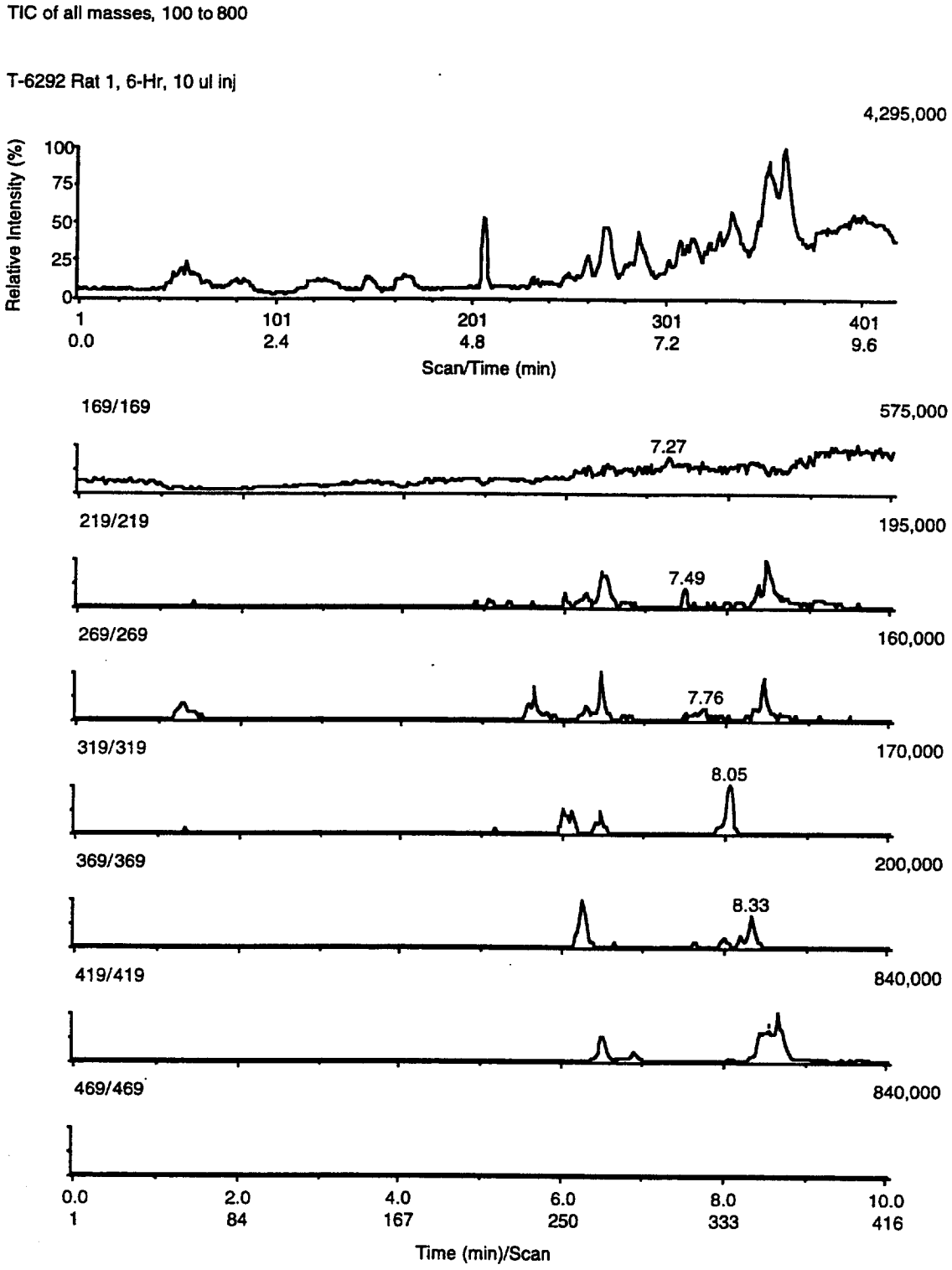


**Figure 12. Extracted ion chromatograms of putative glucuronide peaks for T-6292 homologous series in Rat 1, 6-Hr sample.**

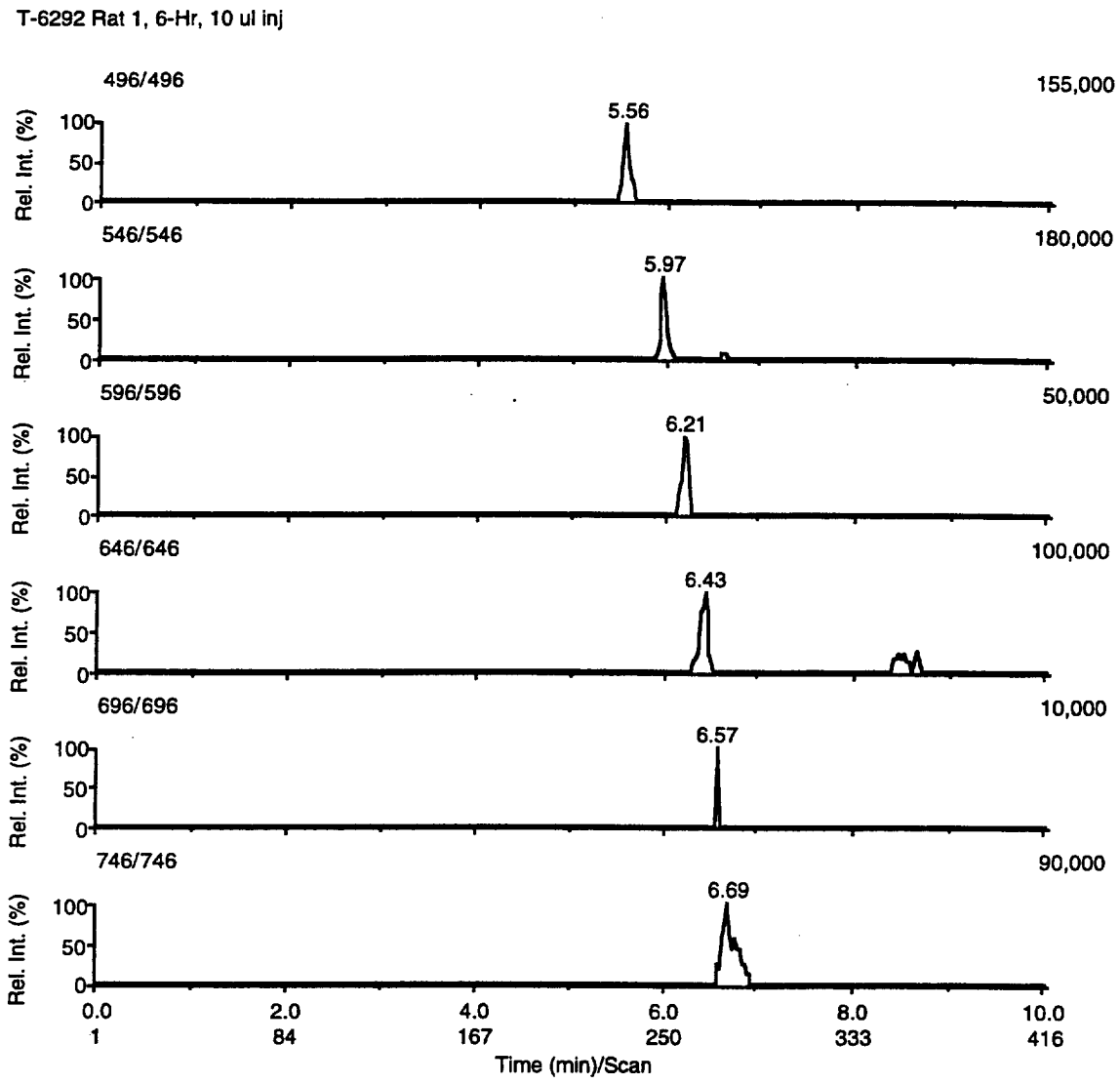


**Figure 13. Extracted ion chromatograms of putative glucuronide peaks for T-6292 homologous series in Rat 1, 0-Hr sample.**

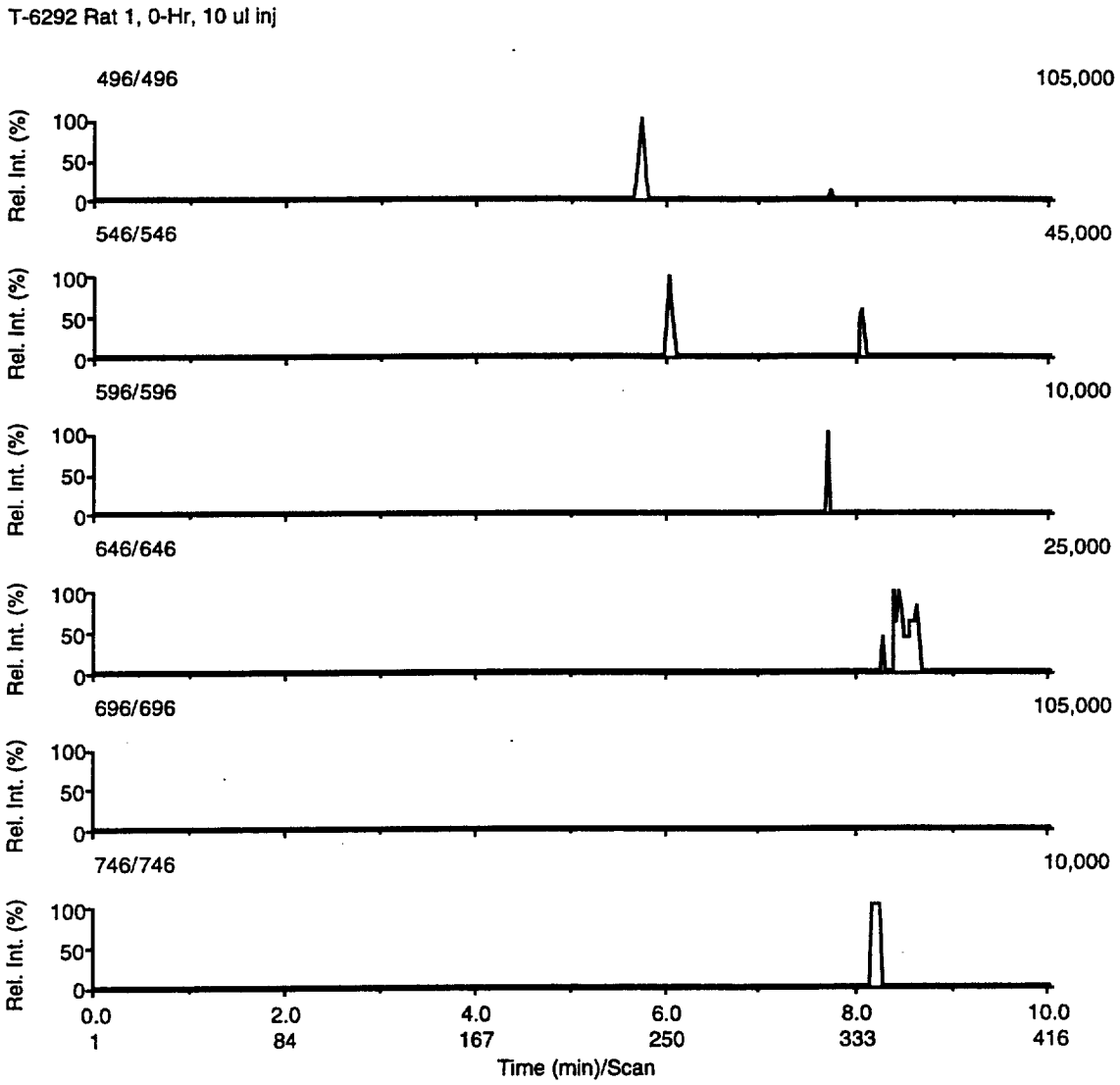
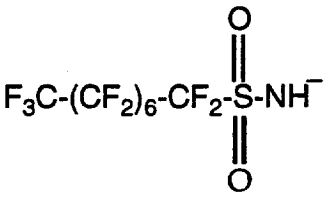


**Figure 14. Proposed structures of the anions of the sulfonamide and carboxylic acid metabolites for T-6292 from Rat 1.**

Sulfonamide Metabolite (m/z 498)



Carboxylic Acid Metabolite (m/z 584)

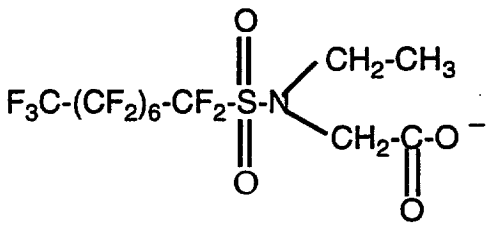


**Figure 15. Extracted ion chromatograms of sulfonamide (m/z 498) and carboxylic acid (m/z 584) metabolites for T-6292 from Rat 1, 0-Hr (top) and 6-Hr (bottom) samples.**

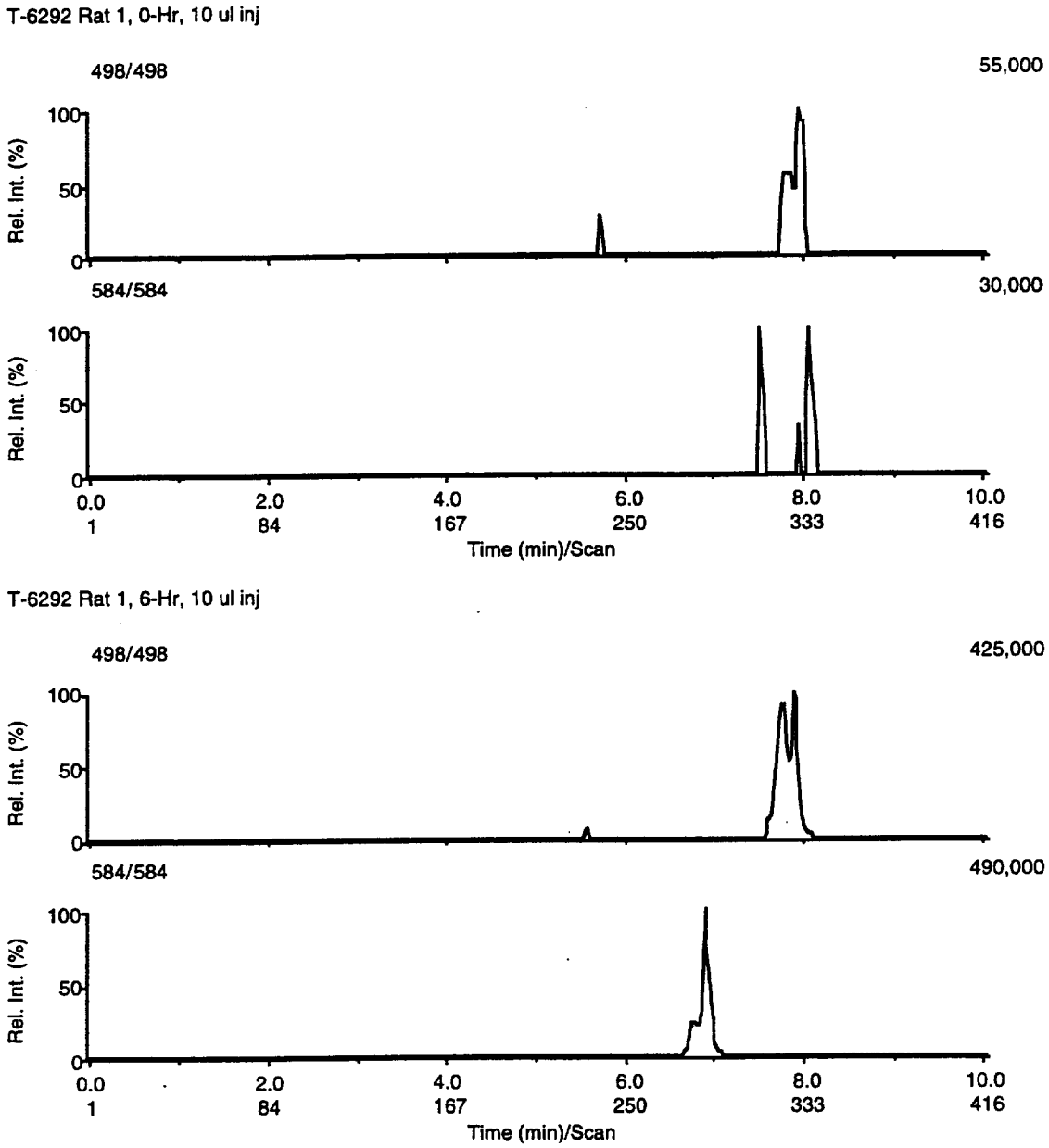
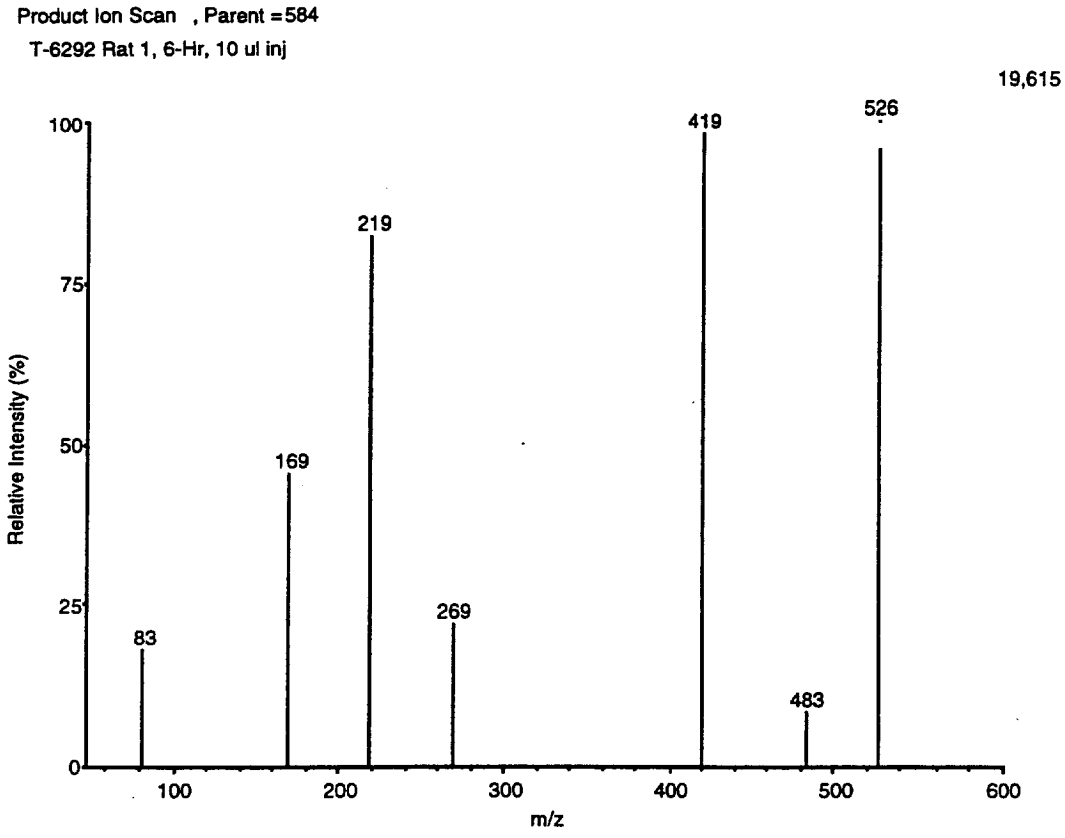


**Figure 16. Product ion scan for carboxylic acid metabolite of T-6292 in Rat 1, 6-Hr sample and proposed fragment structures.**




Carboxylic Acid Metabolite Product Ion Assignments

| m/z (amu) | Description |
|---|---|
| 169, 219, 269, 419, 526 | See Figure 5 |
| 483 | $F_3C\text{-}(CF_2)_6\text{-}CF_2\text{-}SO_2^-$ |
| 83 | $F\text{-}SO_2^-$ |

**Figure 17. Total ion and extracted parent (m/z 650) and proposed metabolite ion chromatograms for T-6293 obtained from Rat Control Sample 13.**

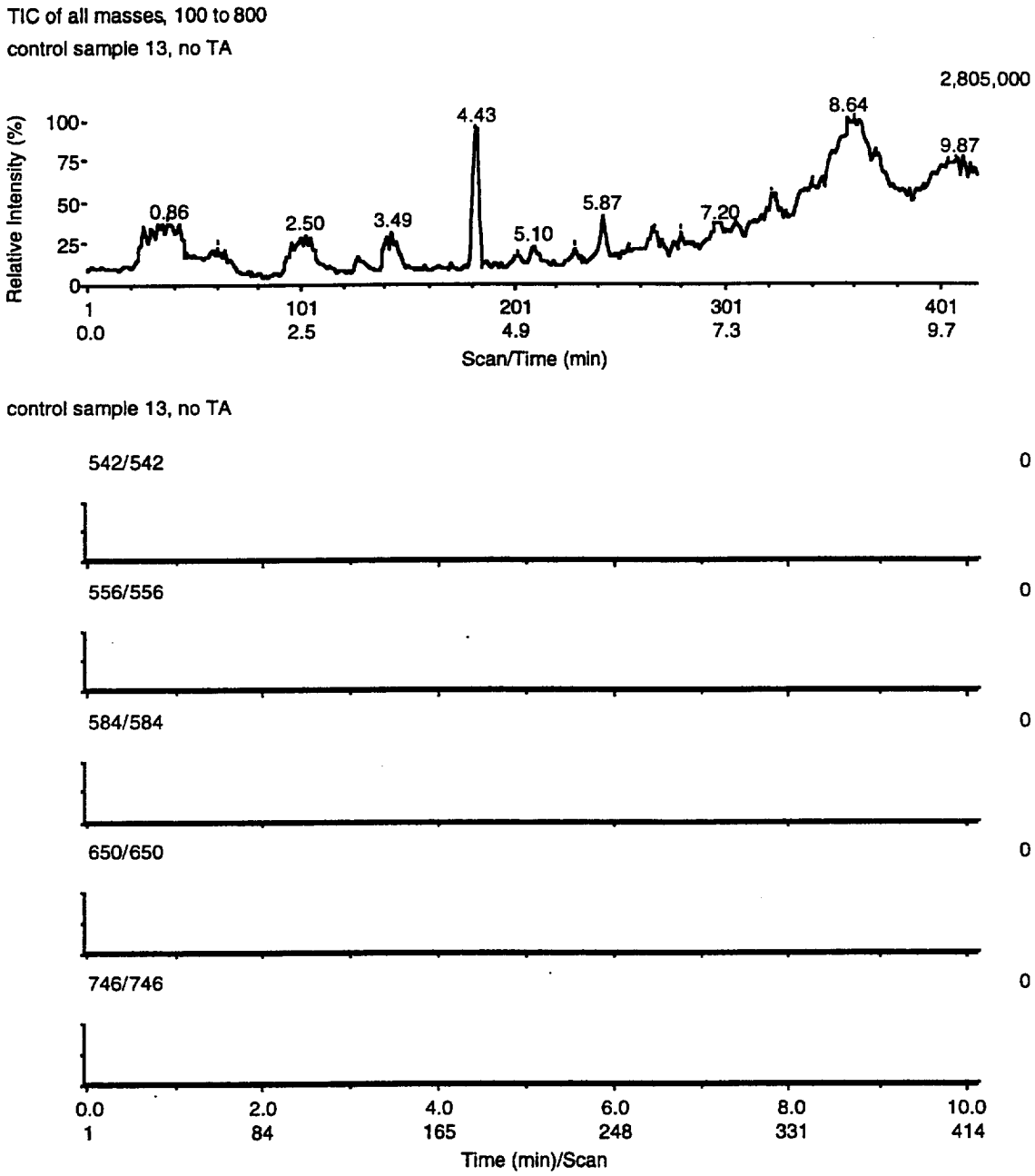


**Figure 18. Total ion and extracted parent (m/z 650) and proposed metabolite ion chromatograms for T-6293 obtained from Rat 1, 0-Hr sample.**

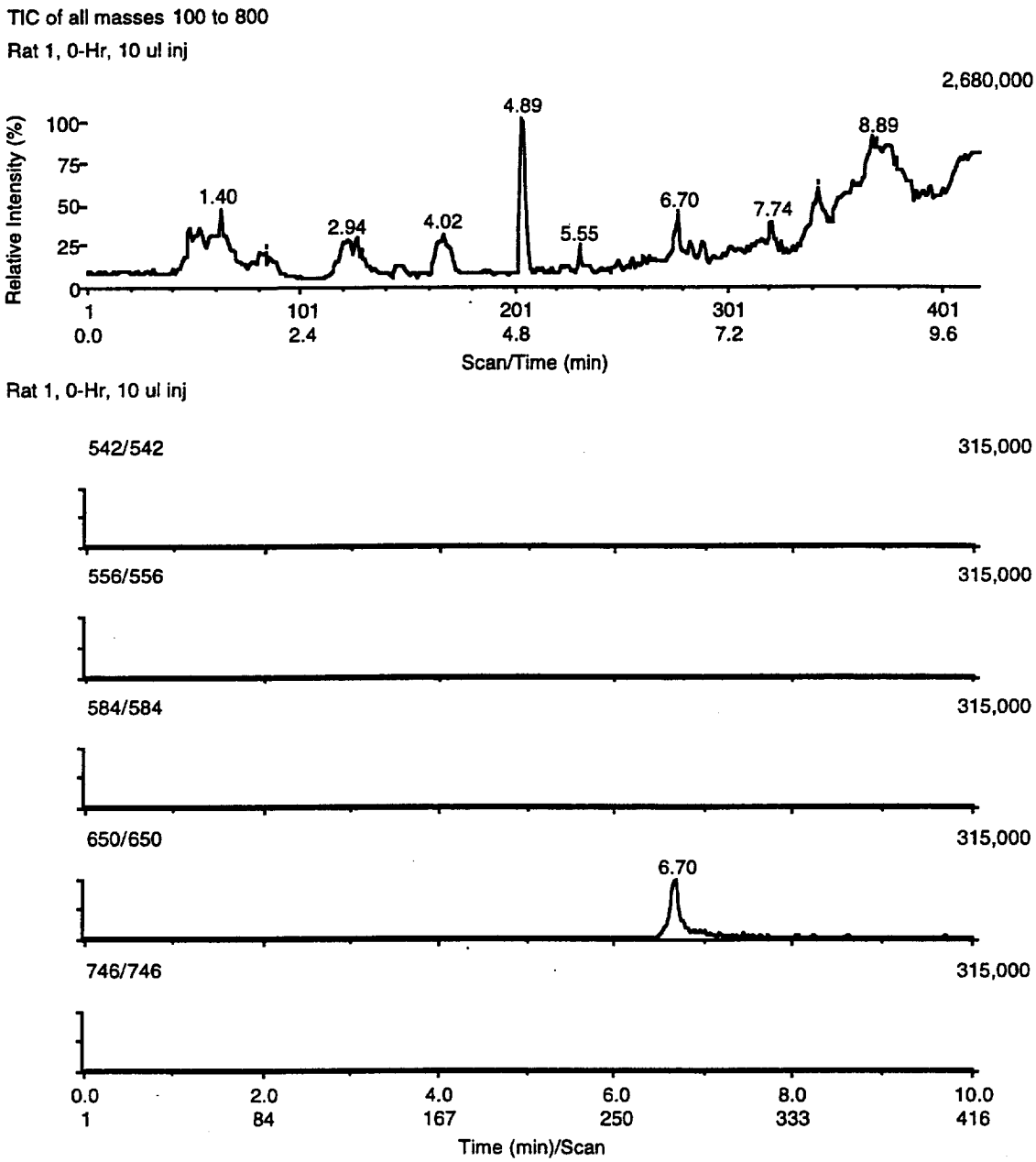


**Figure 19. Total ion and extracted parent (m/z 650) and proposed metabolite ion chromatograms for T-6293 obtained from Rat 1, 6-Hr sample.**

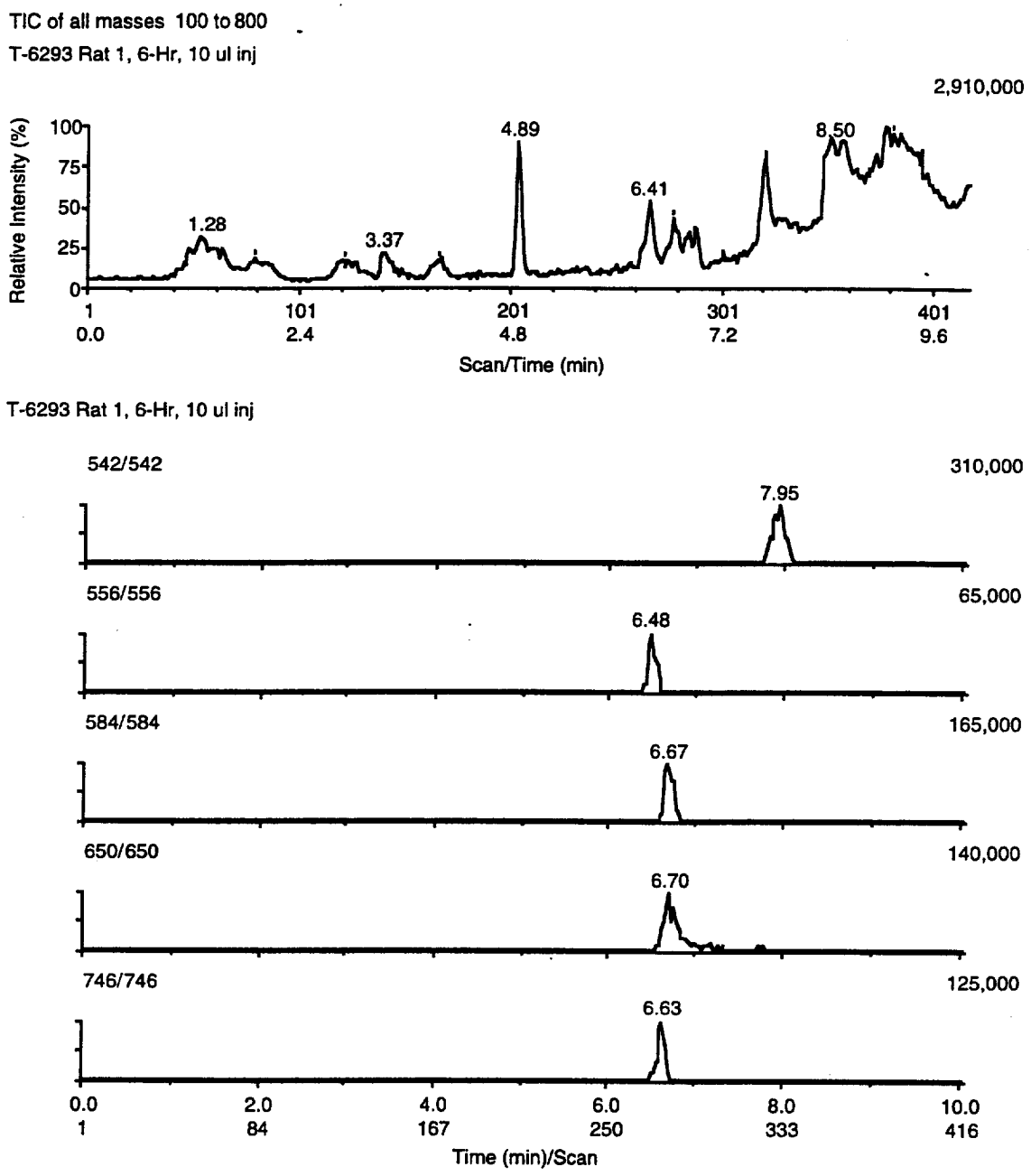
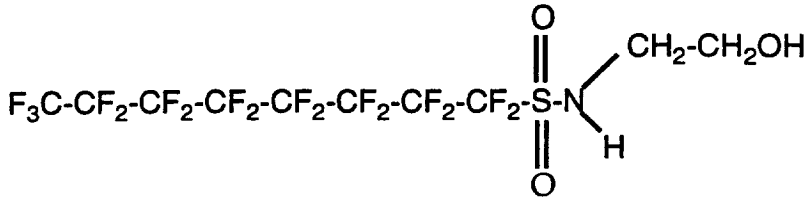


**Figure 20. Possible structures for the observed metabolites of T-6293 with [M-H]⁻ of 542 and 556 amu from Rat 1.**

$M_r = 543$
$[M-H]^- = 542$




$M_r = 557$
$[M-H]^- = 556$

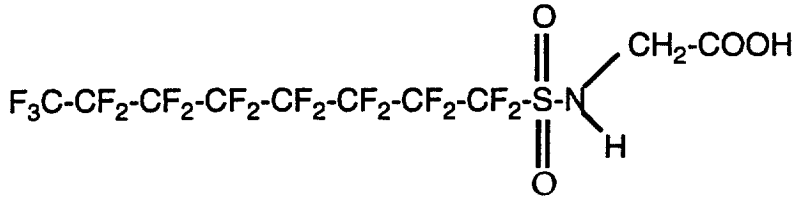
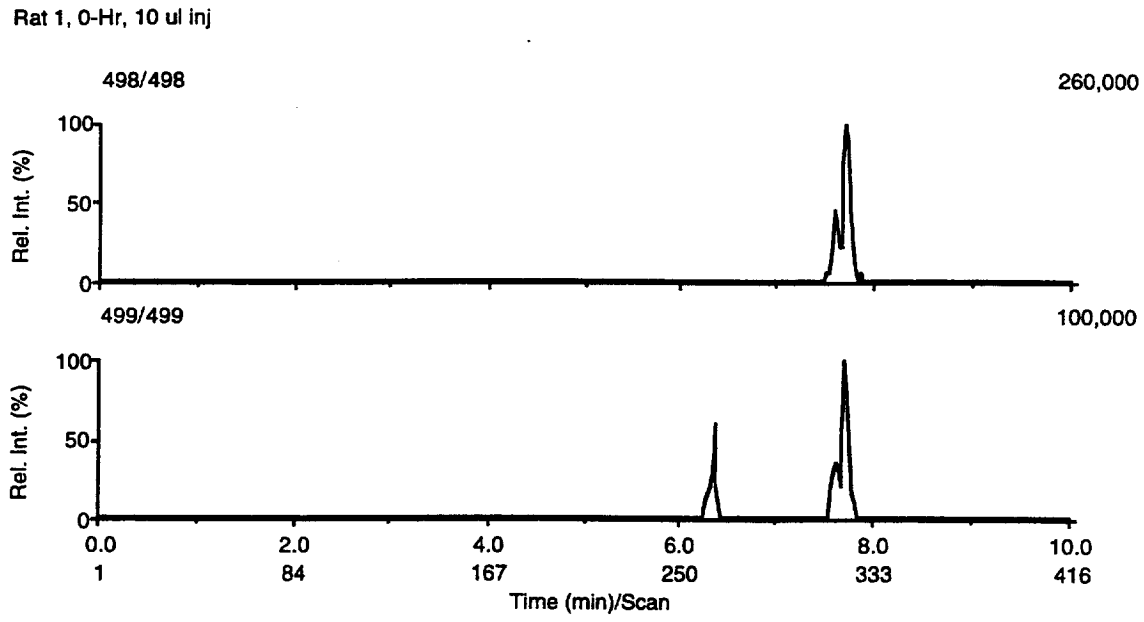


**Figure 21. Extracted ion chromatograms of sulfonamide (m/z 498) and sulfonate (m/z 499) ions for T-6293 from Rat 1, 0-Hr (top) and 6-Hr (bottom) samples.**



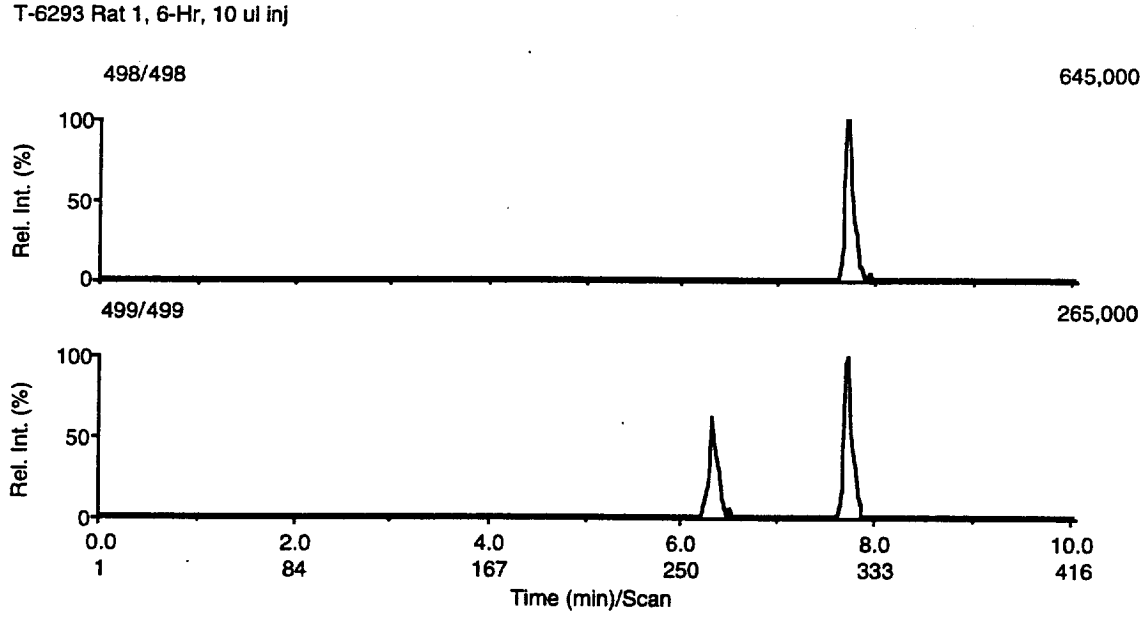
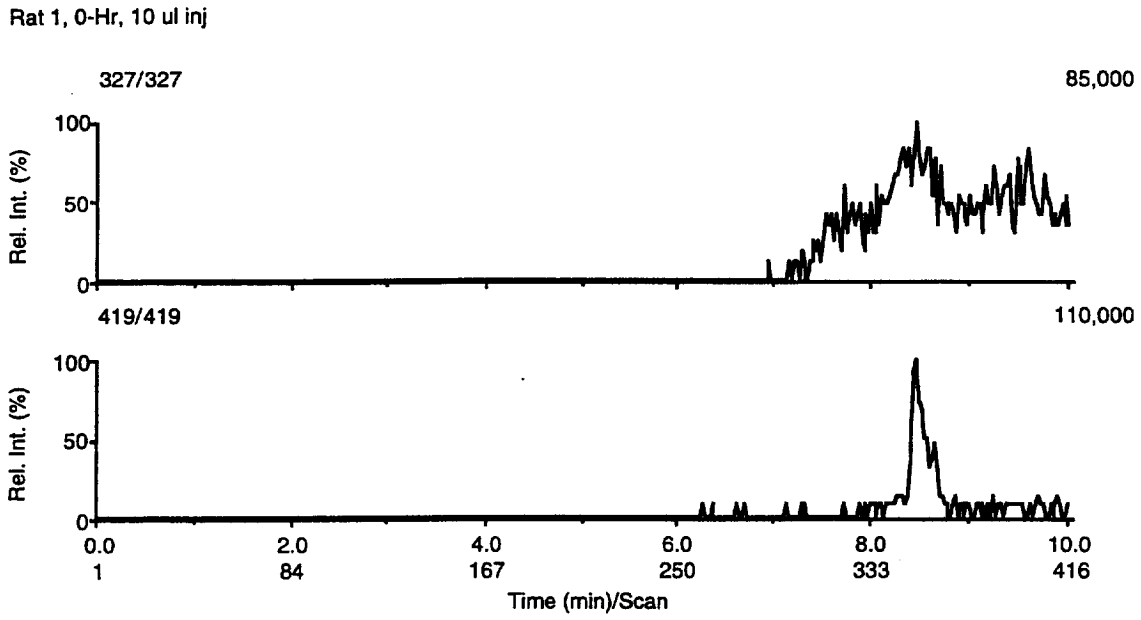



**Figure 22. Extracted ion chromatograms of proposed metabolites with m/z 327 and 419 for T-6293 from Rat 1, 0-Hr (top) and 6-Hr (bottom) samples.**



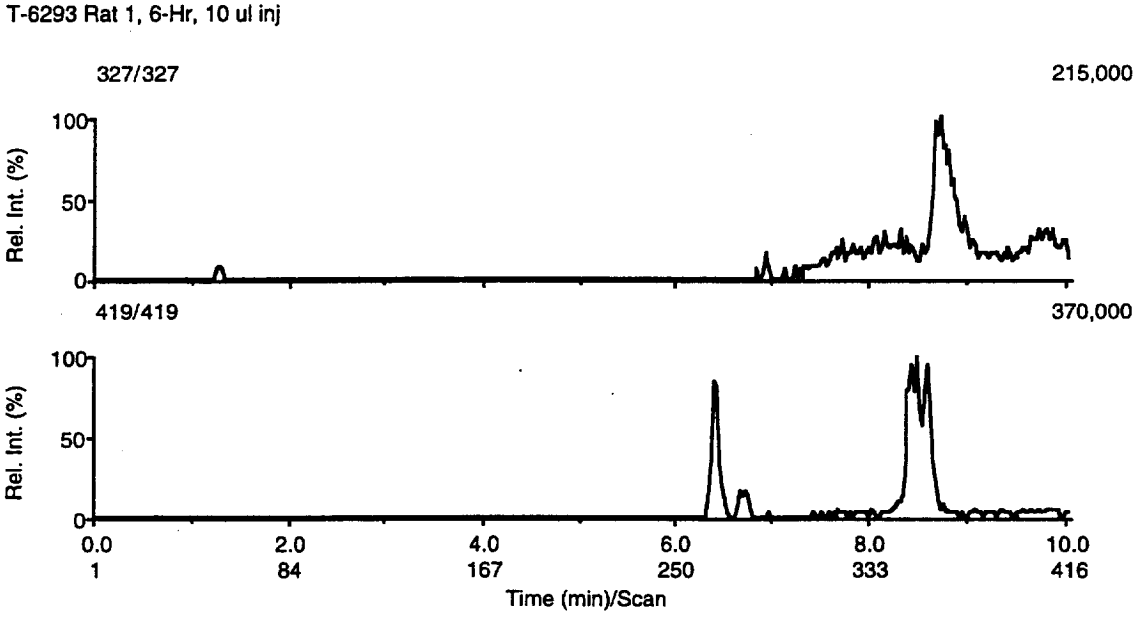



**Figure 23. Total ion and extracted parent (m/z 526) and proposed metabolite ion chromatograms for T-6294 obtained from Rat Control Sample 13.**

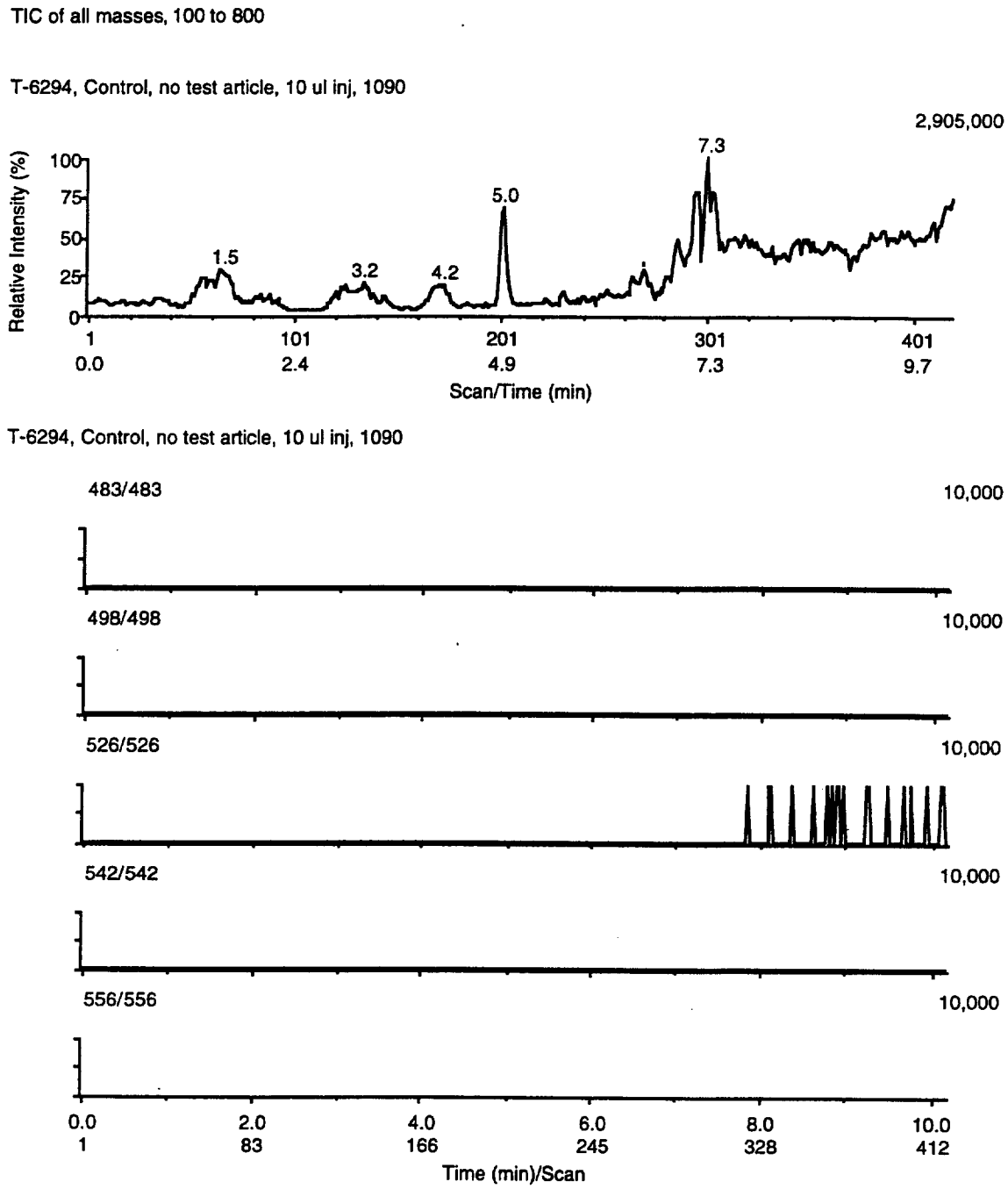


**Figure 24. Total ion and extracted parent (m/z 526) and proposed metabolite ion chromatograms for T-6294 obtained from Rat 1, 0-Hr sample.**

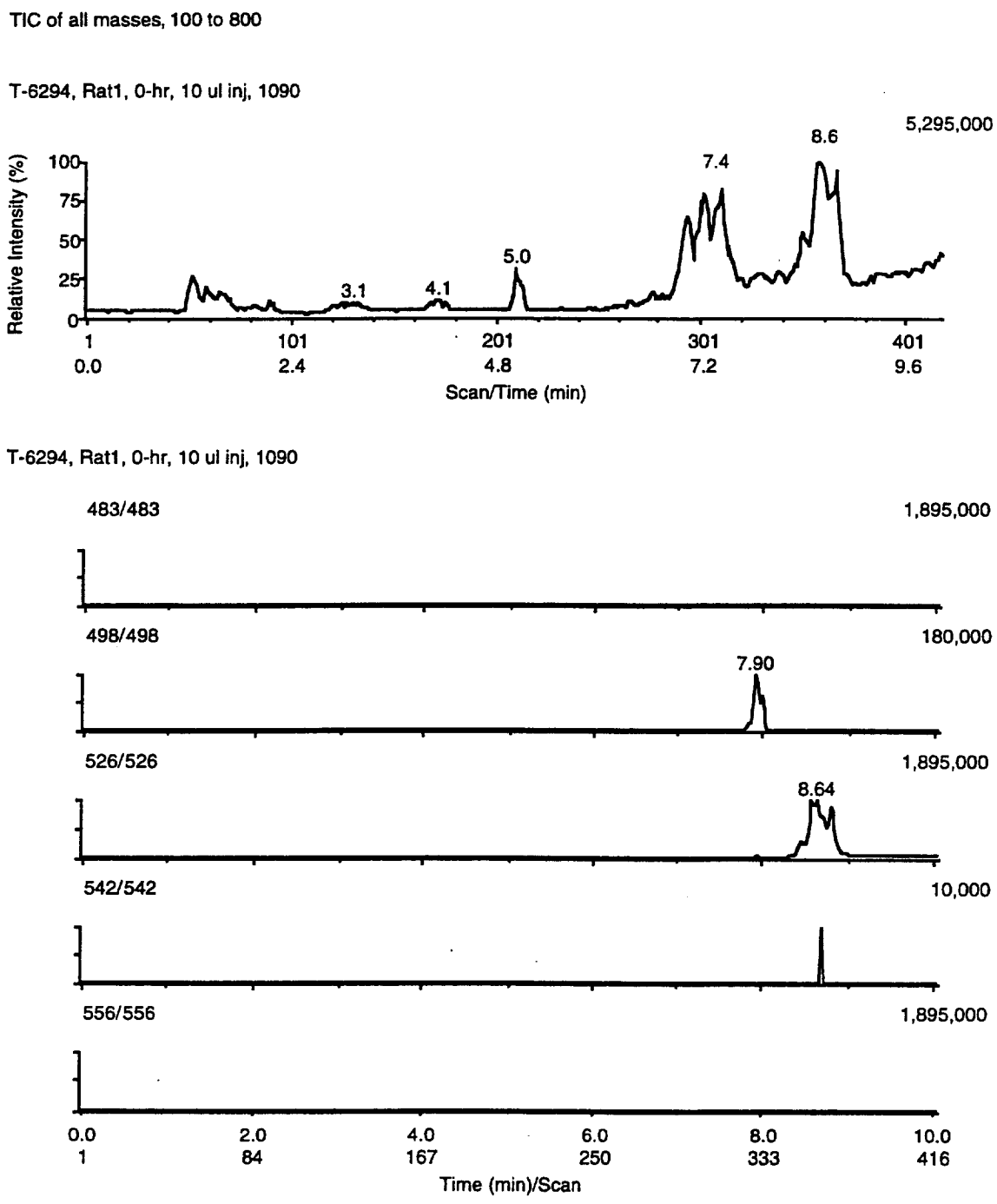


**Figure 25. Total ion and extracted parent (m/z 526) and proposed metabolite ion chromatograms for T-6294 obtained from Rat 1, 6-Hr sample.**

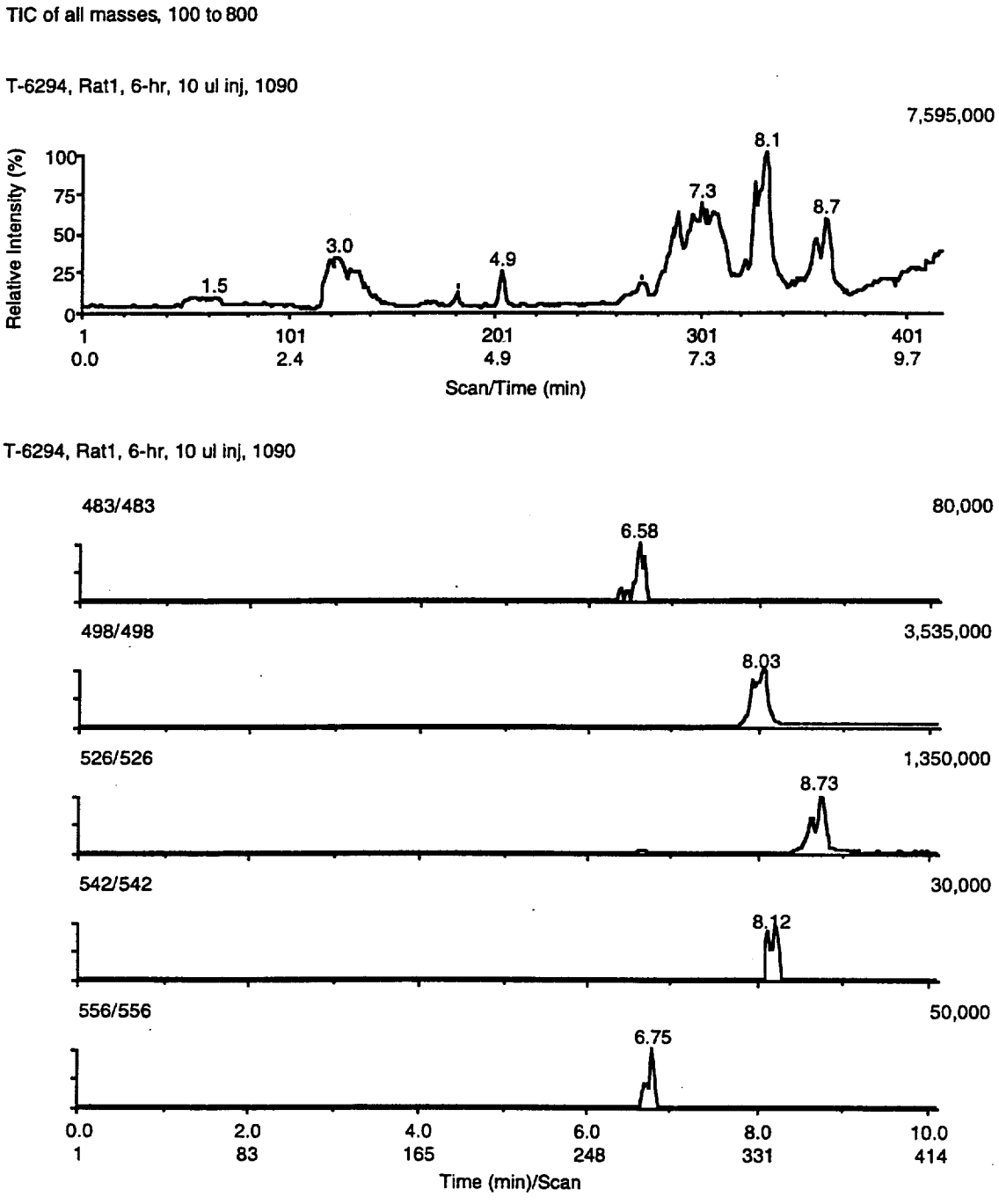
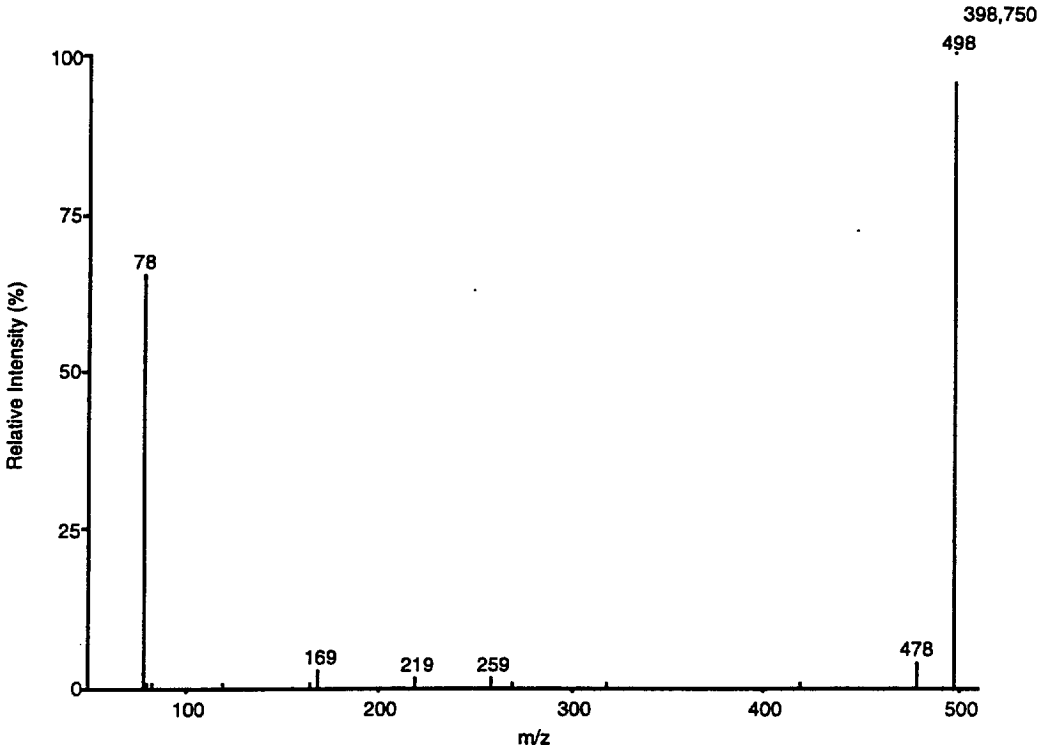


**Figure 26. Product ion scan for perfluorooctanesulfonamide metabolite ($CF_3(CF_2)_7SO_2NH_2$, m/z 498) of T-6294 in Rat 1, 6-Hr sample and proposed fragment structures.**




N-Dealkylated Metabolite Product Ion Assignments

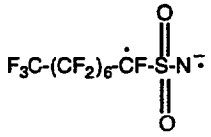
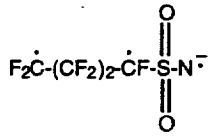
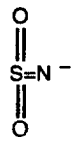
| m/z (amu) | Description |
|---|---|
| 169, 219 | See Figure 5 |
| 478 |  |
| 259 |  |
| 78 |  |

**Figure 27. Total ion and extracted parent (m/z 499) and homolog ion chromatograms for T-6295 obtained from Rat Control Sample 13.**

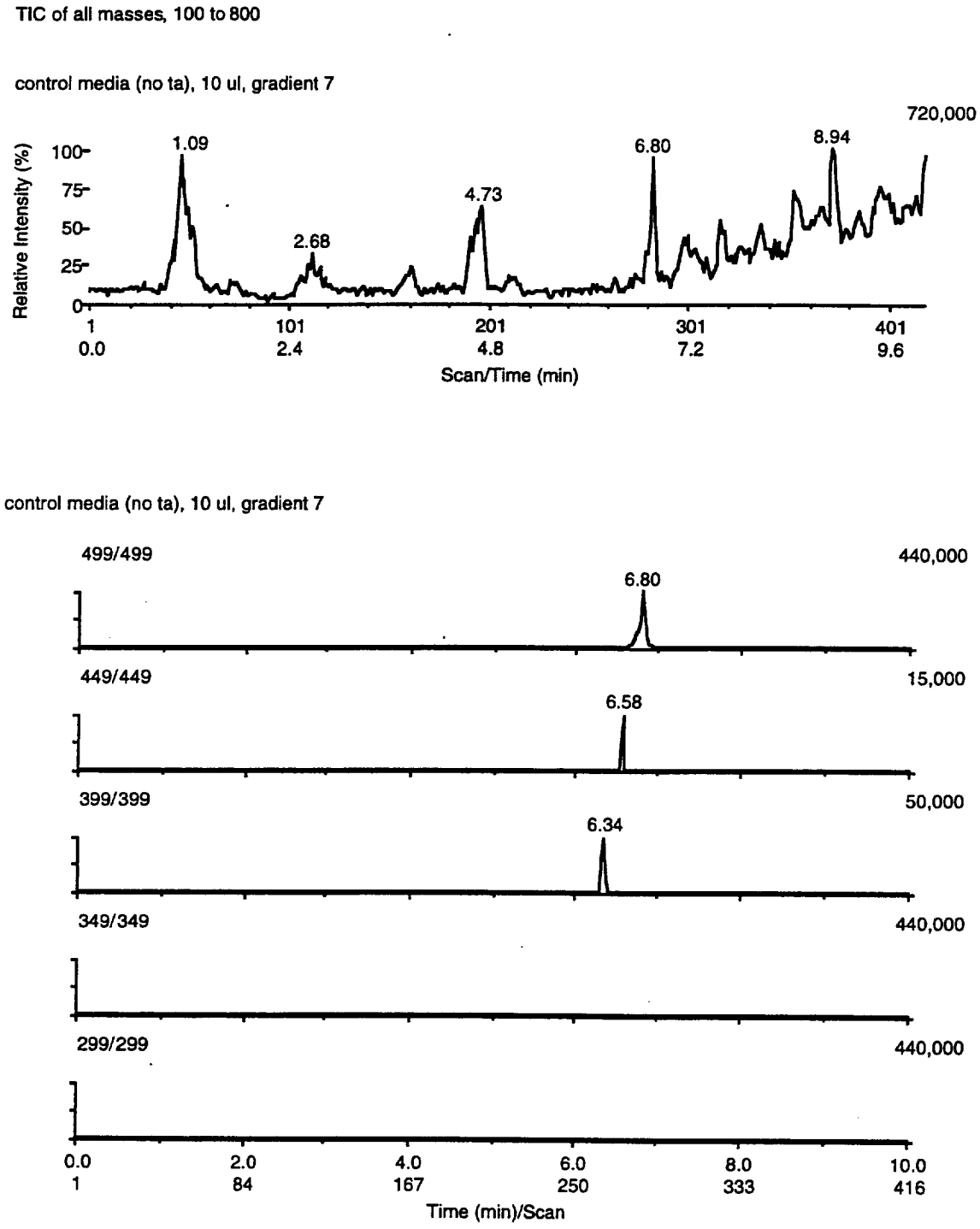


**Figure 28. Total ion and extracted parent (m/z 499) and homolog ion chromatograms for T-6295 obtained from Rat 1, 0-Hr sample.**

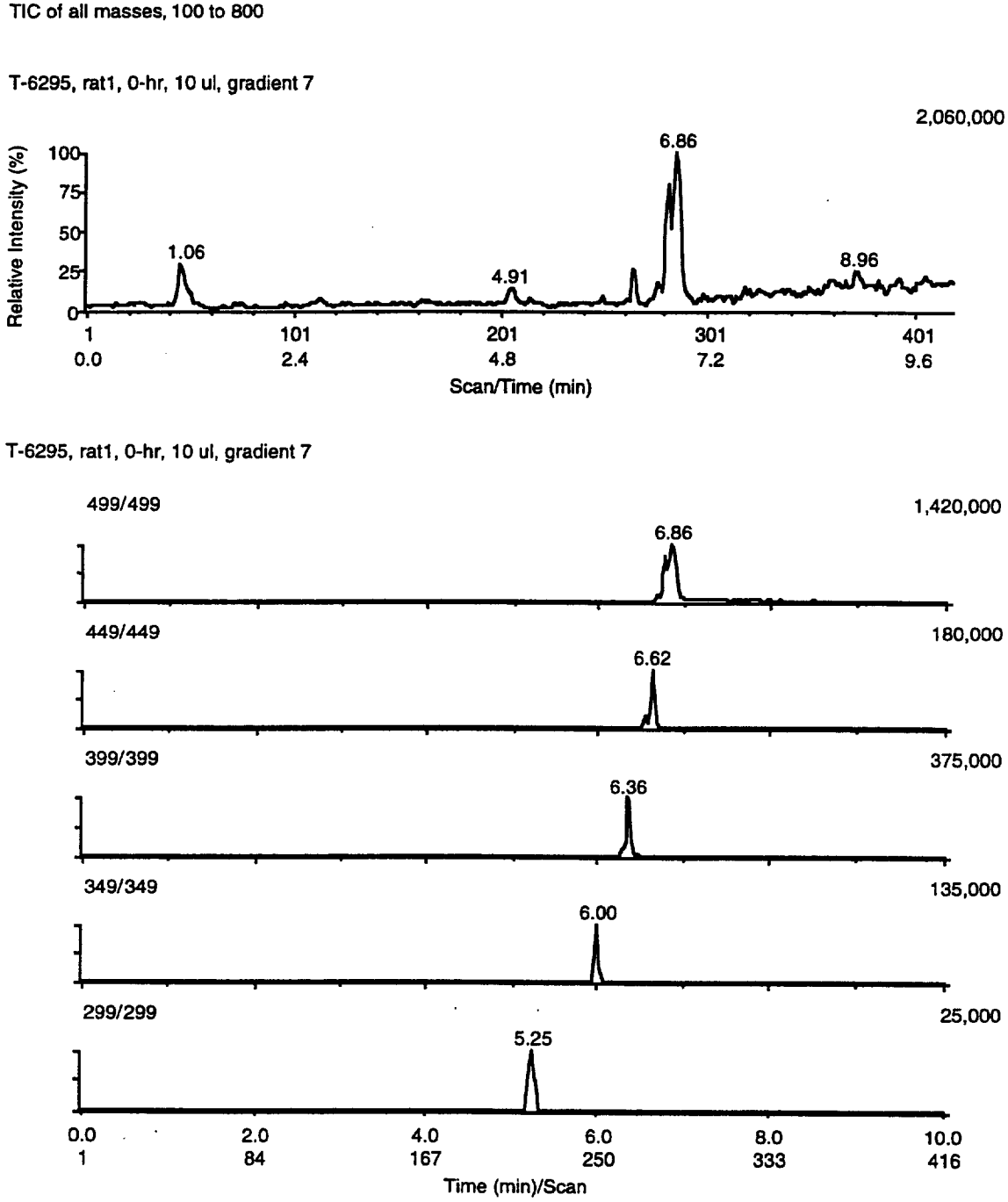


**Figure 29. Total ion and extracted parent (m/z 499) and homolog ion chromatograms for T-6295 obtained from Rat 1, 6-Hr.**