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Forensic Toxicology Research and Development

Evaluation of New and Novel Direct Sample Introduction, Time of Flight Mass Spectrometry (AccuTOF-DART) Instrument for Postmortem Toxicology Screening

Final Report

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ABSTRACT

The National Institute of Justice (NIJ) has sought research and development projects to improve the specificity and sensitivity of analytical tools and technologies used in forensic toxicological analyses. Specifically, NIJ seeks to identify and evaluate tools and technologies that promote faster, more widely applicable, more rugged, less expensive, and less labor-intensive sample analyses. The primary goal of this project was to evaluate a novel direct sample introduction time of flight (TOF) mass spectrometer (JEOL USA, Inc. AccuTOF-DART) for screening postmortem toxicology cases. Early data indicate that this technology has advantages for postmortem toxicology analyses—minimal sample preparation (saving labor and improving sample integrity) and a broader ability to detect a range of compounds than is currently available from most technologies. We evaluated the instrument's ability to detect 112 different drugs and metabolites of interest in postmortem toxicology from methanolic standard solutions. We found that most drugs could be detected, but that detection limits were too high to see likely therapeutic levels of many drugs. We examined these compounds in urine as well and again found that many drugs were detectable, but that detection limits were not sufficient for concentrations encountered. In blood and tissues, a similar pattern was observed. We encountered interferences from isomers, which cannot be distinguished by exact mass, possibly unresolved compounds affecting mass determination, and other matrix components interfering with ionization (e.g., creatinine). Even with sample preparation, sample handling and sample analysis were very rapid.

We also evaluated an autosampler (courtesy of LEAP Technologies) that greatly aided sample handling and contributed to the consistency of results. The JEOL, USA Inc. AccuTOF-DART is a well-constructed instrument that is easily maintained and very robust. The software for the instrument (Mass Center) functions poorly. It is unstable and does not perform in a standard Windows fashion. It is awkward to use and is problematic for forensic handling of data.

Overall, the instrument is promising because the very rapid sample analysis and potential broad sensitivity merit efforts to improve sensitivity and the software. The instrument is currently useful for rapid screening of samples for which sensitivity is not a concern, such as drug chemistries. Confirmatory data would be inconsistent, due to issues of isomer identification. Capability for screening in biological samples is currently limited because of sensitivity. Further work to improve the instrument's sensitivity and software could result in a very useful instrument for biological samples and other applications.

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EXECUTIVE SUMMARY

Forensic pathology relies heavily on forensic toxicology in death investigation. Processing death investigation cases often requires significant expense because of necessary toxicological testing. In addition, comprehensive toxicology analyses can take weeks to months to complete, which can significantly slow the handling of death investigation cases. Slowness in processing delays the signing of death certificates, which delays investigations, criminal prosecutions, and payment of death benefits to survivors. Currently, postmortem toxicology analyses rely predominantly on immunoassays, gas chromatography/nitrogen phosphorous detection (GC-NPD) and color-reaction tests for screening samples to determine if drugs or toxins are present. Initial results are then confirmed for identity and concentration using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). Presently, no single assay is able to detect the hundreds of compounds of interest in a death investigation with the chemical specificity of mass spectral analysis. As a result, an analyte significant to determining the cause and manner of death can go undetected if the proper screening tool is not used.

The scientific literature is filled with case reports of extraordinary efforts and fortuitous circumstances that helped detect a significant compound that otherwise would have escaped detection. Technologies that provide broad spectrum detection are tremendously important to improved resolution and sensitivity in postmortem toxicology analyses. In addition, technologies that reduce sample preparation time are more efficient and prevent errors that may be introduced by extensive sample preparation techniques. The ideal screening technologies are those that improve the quality of the result and increase throughput to address workload demands.

ES.1 Project Goals

The study's purpose was to evaluate the applicability of the direct sample introduction time of flight mass spectrometer (DART-TOF) technology to postmortem toxicology analyses. Specifically, we evaluated the instrument's performance as a screening technology for detecting toxicologically relevant drugs and toxins and to determine if the instrument could potentially improve the efficiency of biological sample screening. We began with drugs of interest in postmortem toxicology prepared in methanol. After evaluating what we were able to see from standard materials, we evaluated sample analyses and preparatory techniques for urine, blood, and tissue. In addition to the evaluation of manufactured materials, we tested previously confirmed postmortem case materials (obtained from Offices of the Chief Medical Examiner (OCMEs) in Chapel Hill, NC; Seattle, WA; and Maricopa County, AZ).

In addition to what was originally proposed for the project, we were able to evaluate the use of an autosampler for the instrument courtesy of LEAP technologies and to directly compare the analysis of two cases on DART-TOF and on five other LC/MS and tandem mass spectrometry (MS/MS) platforms. We also had the opportunity to evaluate the instrument for the analysis and identification of components in illicit cocaine and to compare these results to the Cocaine Signature Program, conducted by the Drug Enforcement Administration (DEA) special testing laboratory, for 25 illicit cocaine materials.

ES.2 Methods

Analytical procedures on the DART-TOF for all stages were similar. Drug standards were supplied by Cerilliant (Round Rock, TX). Polyethylene glycol for mass calibration was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol was purchased from AAPER Alcohol and Chemical Co (Shelbyville, KY), methanol and isopropanol from VWR (West Chester, PA), and acetone from Fisher Scientific (Fair Lawn, NJ). All were high-performance liquid chromatography (HPLC) grade. Glass melting point tubes were supplied by JEOL USA, Inc.

The DART-TOF (JMS T100LC) was purchased from JEOL USA, Inc. (Peabody, MA).The analyses were conduced in both positive and negative modes of the DART ion source. The ion source was operated with a ring lens voltage of 5V, an orifice 1 voltage of 20V, and an orifice 2 voltage of 5V. Electrodes 1 and 2 of the source were set to 150V and 350V, respectively, while the temperature was set to 300°C. The detector for this particular system was optimized at 2,200V. The system was mass calibrated using polyethylene glycol before each sample run. Samples were introduced into the analyzer in one of two ways, either by manual introduction or by introduction with the autosampler. The solutions of stock methanolic drug standard were introduced by dipping the glass probe into the sample and passing it through the ion stream.

The project was divided into four stages.

ES.2.1 Stage I: Analysis of Drugs in Standards

Pure drug standards were used in this stage. We started with concentrated solutions of model drugs for classes of pharmaceuticals (e.g., benzodiazepines, atypical antidepressants, stimulants, tricyclic antidepressants, serotonin reuptake inhibitors). Particular attention was paid to drugs for which current screening methods are particularly weak. The total number of compounds examined was 112.

ES.2.2 Stage II: Analysis of Drugs in Urine

After optimization of the instrument for the detection of pure solutions of standards in Stage I, we evaluated the instrument's performance with drug compounds (e.g., representative analogs) spiked into a urine matrix. The drugs chosen were determined in part based on the drug content of routine postmortem cases. Concentrations used and the drugs analyzed were tailored from the information gathered in Stage I.

We evaluated limits of detection (LOD) by diluting solutions until the peak of interest was no longer identifiable either by deviating more than 8–10 mmu from the theoretical monoisotopic mass or the peak was not discernible from the background. This provided an assessment of the technology's applicability to other analytes in the same structural class.

We first evaluated the ability of the instrument to detect target compounds in neat urine (i.e., no extraction or other sample preparation treatments). Based upon these results, we examined the effect of a simple organic solvent extraction and concentration. The goal was to achieve screening results with minimal sample preparation. A requirement for extensive sample preparation (e.g., solid-phase extractions and/or back extractions and significant concentration or derivatization steps) would significantly limit the instrument's application in postmortem toxicology work. Stock drug standards (1 mg/mL) were diluted into the appropriate volume of blank urine to prepare 100 μg/mL of working standard solutions. The working solutions were serial diluted until LOD was reached. We then evaluated the instrument's performance with postmortem urine samples from archived cases.

ES.2.3 Stage III: Analysis of Drugs in Blood and Tissue

In these studies, drugs were spiked into human blank blood. The blood was hemolyzed using sodium fluoride and sodium chloride (a common preservative found in "grey top" blood collection tubes). Early evidence indicated that blood sample analysis requires precipitation of proteins. We examined a precipitation method with acetonitrile. We evaluated whether concentration of the sample could be easily achieved in this preparation, and then we evaluated the instrument's performance with postmortem blood samples and tissues from archived cases in a manner similar to that used for urine.

We also analyzed numerous archived postmortem cases. **Table ES-1** gives a distribution of the number and types of cases analyzed. Correlation of screening results obtained with the DART-TOF system to those obtained in the original analyses of these cases was also case dependent, given that the original

analyses of these types of samples were likely problematic. Typically, matrices such as these were difficult to analyze originally, and interpretation of the correlation between the DART-TOF system and the original analysis was cautious. The list of drugs was determined in part by the drugs present in the postmortem cases available. **Table ES-2** provides a list of the drugs in tissue and blood that were previously reported in the cases analyzed.

Table ES-1. Distribution of the Number and Types of Cases Analyzed

Specimen	Cases Analyzed
Blood	28
Liver	11
Kidney	2
Brain	4

Table ES-2. List of the Drugs in Tissue and Blood Analyzed

Acetaminophen	Dextromethorphan	Morphine
Alprazolam	Diazepam	Nicotine
Amphetamine	Diphenhydramine	Nortriptyline
Atropine	EME	Olanzapine
Benzoylecgonine	Fluoxetine	Oxcarbazepine
Caffeine	Fluvoxamine	Oxycodone
Carbamazepine	Gabapentin	Oxymorphone
Carisoprodol	Hydrocodone	Paroxetine
Chlopheniramine	Ibuprofen	PCP
Citalopram	Levorphanol	Promethazine
Clonazepam	MDMA	Propoxyphene
Cocaine	Methamphetamine	Quetiapine
Codeine	Methadone	Temazepam
Cotinine	Methylphenidate	THC
Cyclobenzaprine	Meprobamate	Topiramate
Desmethylcitalopram	Metoprolol	Trazodone
Desmethyldoxepin	Mirtazapine	

We also analyzed several cases after solid-phase extraction. We were able to compare the analysis of these samples (one cocaine case and one methadone case) to those of several other mass spectral platforms. These samples were analyzed on a consistent LC system with five separate MS and MS/MS platforms for detection.

ES.2.4 Stage IV: Additional Study Comparison of Cocaine Analysis with DEA

Twenty-five illicit cocaine hydrochloride samples were obtained from the National Institute of Drug Abuse's Division of Neuroscience & Behavioral Research (Bethesda, MD). Cocaine analyte standards (e.g., cocaine, anhydroecgonine methyl ester, benzoylecgonine, cocaethylene, norcocaine) were purchased as hydrochloride salt (1 mg/mL) or base solutions of methanol (benzoylecgonine) or acetonitrile from Cerilliant (Austin, TX). These samples were analyzed by the DEA Special Testing

Laboratory (courtesy of John Casale) and Armed Forces Institute of Pathology (AFIP), and these results were compared to those obtained with the DART-TOF.

While the evaluations of these components were not separate stages, throughout the project we noted how the instrument performed and how the autosampler which was provided by LEAP Technologies early in the project was helpful. We also noted how the software performed. We discuss our impressions of these components in Section 3.6, Discussion of the Equipment and Software Performance.

ES.3 Results

ES.3.1 Stage I: Analysis of Standards

Of the 112 compounds tested, 4 compounds were not detected from the original list. These four were buprenorphine-glucuronide, 9-carboxy-11-nor- tetrahydrocannabinol, 9-carboxy-11-nor-D9 THC-glucuronide, and psilocybin. While buprenorphine was not detected, the buprenorphine and glucuronolactone could be detected. All other compounds tested produced an expected M+H ion. Forty-nine compounds tested did not produce an expected M-H ion. As well as producing the M+H ion, many compounds also produced a dimer +H of the compound in positive mode. Dimer ions can also be useful in determining an unknown compound. Mass accuracy was very good for most compounds; however, mass accuracy was unpredictably variable for a few compounds for undetermined reasons. Mass accuracy can be affected by sample introduction dynamics and can pose a challenge. Spectra of all compounds analyzed are presented in **Appendix A**.

During the analysis, 14 potential isomer or interference pairs were identified. These pairs of compounds were exact isomers or had monoisotopic masses similar enough to be difficult to resolve due to isotopic contributions. These compounds were analyzed using different parameters to fragment each compound for identification and to examine whether characteristic fragmentation could be obtained. The analysis of isomer pairs, such as cocaine and scopolamine, was conducted in positive analysis mode by raising the voltage on the entrance orifice cone (O1) from 5V to 90V in order to produce fragments of each compound. Distinct and reproducible fragments were apparent. In contrast, morphine and norcodeine did not produce consistent fragments. With the exception of morphine and norcodeine, all other isomer pairs were distinguishable by unique ions from fragmentation. While isomers may be distinguishable in isolation, fragmentation in a mixture of a biological matrix is not particularly viable due to the complex collection of masses and subsequent fragmentation of unknown compounds in the sample.

The DART TOF successfully identified 108 compounds commonly analyzed in postmortem forensic testing from manufactured standard solutions. The data obtained from each analysis, including isomer fragmentations, are stored within the instrument's library search program for future comparison. The creation of these libraries enhances the DART-TOF by providing fast and reliable data analysis. Libraries generated in this manner could potentially be shared with other sites to promote standardization of future analyses between forensic laboratories.

ES.3.2 Stage II: Urine Analysis

Urine specimens were also evaluated on the DART-TOF system. While some drugs, such as cocaine and methadone, were detected relatively easy, and the DART-TOF system achieved sensitivities for these drugs that are relevant to postmortem toxicology, overdose cases' appropriate sensitivity for other drugs was not attained. Interferences, such as creatinine, also resulted in lower sensitivity. Mechanisms to separate drugs of interest from creatinine would be necessary to resolve this interference. Unfortunately, this type of separation would also limit some advantages of the instrument because the sample preparation would then be similar to what is needed with other chromatographic methods. Lastly, isomers or compounds that are close in mass may cause interference problems, resulting in identification problems.

While some of these compounds can be identified by fragmentation in isolation, when present in a mixture of an unknown sample, fragmentation is not as viable due to the likely presence of many background peaks. Again, this increases the demand for sample preparation and decreases the advantage of this system over a traditional chromatographic analysis.

ES.3.3 Stage III: Blood and Tissue Analysis

For compounds spiked into blood or in archived samples, direct introduction of the blood into the DART ionization stream did not successfully detect compounds. At the least, minimal drug extraction/precipitation was needed in order to achieve sensitivity for drugs of interest. A sample precipitation with acetonitrile provided improved sensitivity, and further concentration of the supernatant improved sensitivity more. The preparation used served to remove interferences and concentrate the sample. Drugs detected in spiked blood had better sensitivity than in postmortem specimens at the same concentration. This may be due to more extensive protein binding in actual samples or the potential presence of more interference due to the degradation of the samples over time. Overall, sensitivity for drugs was not sufficient for likely concentrations that would be encountered in most cases. This instrument is not ideal for detection of drugs in postmortem samples at therapeutic levels; however, the specimens used in this study were old, and compound degradation may have occurred.

Two cases in particular were extracted using solid-phase extraction methods and were analyzed by both DART-TOF and other mass spectral platforms. We were particularly concerned that previously confirmed drugs were not detected due to the age of the samples. Samples were prepared by solid phase extraction for analysis by all platforms; however, this sample preparation did not necessarily improve the sensitivity on the DART-TOF. These samples were easily detected, and quantitative results were consistent with prior confirmation on more traditional LC/MS and MS/MS platforms. From these data, it was evident that previously reported compounds were readily detected by these LC/MS and LC/MS/MS platforms. **Table ES-3** summarizes the detection and/or quantitation determined on each of the platforms used. Good agreement was obtained between our current quantitations in these cases and the previous quantitative results obtained with LC TOF and LC/Q-TOF. There was evidence in the data collected by LC/Q-TOF, LC/Trap, and LC/TOF of the compounds previously reported in the sample. So, the samples had not degraded in storage, and detection issues on DART-TOF are a result of sensitivity of the DART-TOF.

Table ES-3. Summary of the Quantitative and Detection Results for the Two Cases Run on Multiple MS Platforms

	Case 1			Case 2
Platform	**COC**	**BE**	**CE**	**Methadone**
Original Quant	30ng/ml	1,760ng/ml	ND	1,100 ng/ml
LC/MS	8.8	1253	2.7	1,156
LC/Trap*	Detected	Detected	Detected	Detected
LC/TOF	12.5	1,632	6.4	754**
LC/QTOF	26.1	1,539	10.4	898
LC/QQQ	1.1	1,448	0.1	1,134
DART-TOF	ND	ND	ND	

* No quantitation attempted on the trap

** Source was saturated, resulting in poor quantitation

Note: Quantitations were consistent with original results from traditional platforms; however, the compounds were not detected by DART-TOF.

The DART-TOF analysis of these cases was significantly faster and easier that any of the LC/MS or MS/MS platform analyses, but the comparison did highlight that the sensitivity of the DART-TOF is a significant issue for its use in biological samples.

ES.3.4 Stage IV: Additional Study Comparing Illicit Cocaine Analysis Methods

Of the solvents and adulterants/diluents detected by DEA, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), and dimethylterephalate were identified by the DART-TOF. The DART-TOF allowed for the rapid introduction and analysis of 25 illicit cocaine samples without the need for sample preparation. Although this direct analysis resulted in rapid production of data, it also caused inconsistent results. Since the introductions of the powdered samples were done manually, without the use of an autosampler, the outcome was analyst-dependent. Many samples were analyzed multiple times in an effort to verify the presence or absence of the analytes. Although analytes such as anhydroecgonine methyl ester (AEME) and cinnamoylcocaine were easily detected in most of the samples, others such as tropacocaine and 3,4,5-trimethoxycocaine were minimally detected, if at all.

The DART-TOF is a novel approach to forensic analysis; however, the analysis of crude illicit cocaine in this study proved ineffective for detecting the presence of many compounds that would be used to trace a cocaine sample to its geographic origin. In an effort to increase laboratory production, forensic laboratories could utilize the DART-TOF as a rapid screening test for preliminary sample-to-sample comparison work. This could then be confirmed by a more thorough investigation, such as signature analyses.

Potentially, a similar situation to the creatinine interference in urine may play a roll in the analysis of cocaine in this circumstance. The presence of large quantities of cocaine may interfere with the ability of the lower concentration components to ionize, and, thus, to be detected.

ES.4 Instrument Reliability, Autosampler, and Software Issues

Over the course of the project, we found the DART-TOF instrument required very little maintenance. The instrument has been robust and has maintained its analytical performance well. The autosampler concept from LEAP Technologies was useful in facilitating throughput, reducing technician input and fatigue, and improving analytical reproducibility. Currently, the disposable tips for the autosampler are very expensive, at more than $1 per tip. This cost would likely come down with more units in the field. The tips could be reused successfully because they are very easy to assay for contamination prior to use.

The single most significant issue for the instrument, other than its sensitivity, as demonstrated in the various stages of the study, is the instrument's problematic software, Mass Center, which is a necessary component for running the instrument. The software is unstable and prone to unexpected failure, as well as being non-Windows compliant, even though it appears to be a Windows application. The user experiences unexpected performance relative to other Windows applications (e.g., windows that do not minimize or remain hidden or appear on top of other windows). The software is also very awkward, requiring many multiple windows to be open to run the instrument (we have installed a second monitor to provide enough screen space to use all the windows). Some operations, such as starting a sample run, require many multiple steps to accomplish, and the design of the software precludes effective automation. Additionally, the operation of the software may be ultimately problematic for the forensic presentation of the data obtained. The software has many manual steps and no adequate audit trail capabilities, and it is difficult to trace the relationship of derived data (such as processed spectra) to the original data collection. All of these issues would provide many opportunities to challenge the data presented and result in unnecessary demands to explain the data. We have extensively discussed our concerns about the software with the manufacturer.

The results of this study have been widely disseminated through numerous presentations at national meetings, and they are also available as an on-demand Web-based course. We intend to submit at least one manuscript on the project to the *Journal of Forensic Sciences* for publication.

Further research for the instrument should include more evaluation of the instrument's application to controlled substances, particularly multifacility comparisons to address the reproducibility of the instrument. Also, measures to improve the sensitivity of the instrument would increase its applicability to biological specimens. Finally, the development of spectral libraries for use with identification of complex samples would also increase the applicability of the instrument to many fields.

1. INTRODUCTION

1.1 Statement of the Problem

Forensic pathology relies heavily on forensic toxicology in death investigation. Processing death investigation cases often requires significant expense because of necessary toxicological testing. In addition, comprehensive toxicology analyses can take weeks to months to complete and can significantly slow the handling of death investigation cases. Slowness in processing delays the signing of death certificates, which delays investigations, criminal prosecutions, and payment of death benefits to survivors. Currently, postmortem toxicology analyses rely predominantly on immunoassays, gas chromatography/nitrogen phosphorous detection (GC-NPD), and color reaction tests for screening samples to determine if drugs or toxins are present. Initial results are then confirmed for identity and concentration using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). Presently, no single assay is able to detect the hundreds of compounds of interest in a death investigation. As a result, an analyte significant to determining the cause and manner of death can go undetected if the proper screening tool is not used.

The scientific literature is filled with case reports of extraordinary efforts and fortuitous circumstances that helped detect a significant compound that otherwise would have escaped detection. Technologies that provide truly broad spectrum detection are tremendously important to improved resolution and sensitivity in postmortem toxicology analyses. In addition, technologies that reduce sample preparation time are more efficient and prevent errors that may be introduced by extensive sample preparation techniques. The ideal screening technologies are those that improve the quality of the results and increase throughput to address workload demands.

1.2 Literature Citations and Review

Time of flight (TOF) mass spectrometry using exact mass determination has the potential to greatly improve postmortem screening (Song et al., 2004 Ojanpera et al., 2005; Laks et al., 2004; Cody et al., 2005). A new mass spectral technology using this instrumental theory was recently released and has the potential to simplify sample preparation and provide broad-range sensitivity. JEOL USA, Inc. (Peabody, MA) has introduced a system called DART-TOF. The direct analysis in real time (DART) ion source, coupled with a TOF mass spectrometer, potentially allows for postmortem samples to be easily and rapidly screened in a non-destructive fashion for a wide range of compounds with little sample preparation. Both sample preparation and sample screening for multiple drug analytes can possibly be completed in minutes with the DART-TOF, whereas conventional screening techniques may take up to 8 hours or longer. The system provides sufficient selectivity and accurate elemental composition assignment through exact mass determination. These properties should provide an assay for a wide variety of small molecules, such as drugs and toxins, with minimal sample preparation.

DART is based on the atmospheric pressure interactions of long-lived electronic excited-state atoms or vibronic excited-state molecules with the sample and atmospheric gases. The DART ion source is shown in **Figure 1-1**. A gas (typically helium or nitrogen) flows through a chamber where an electrical discharge produces ions, electrons, and excited-state (metastable) atoms and molecules. Most of the charged particles are removed as the gas passes through perforated lenses or grids, and only the neutral gas molecules, including metastable species, remain. A perforated lens or grid at the exit of the DART provides several functions: (1) it prevents ion-ion and ion-electron recombination, (2) it acts as a source of electrons by surface Penning ionization, and (3) it acts as an electrode to promote ion drift toward the orifice of the mass spectrometer's atmospheric pressure interface.

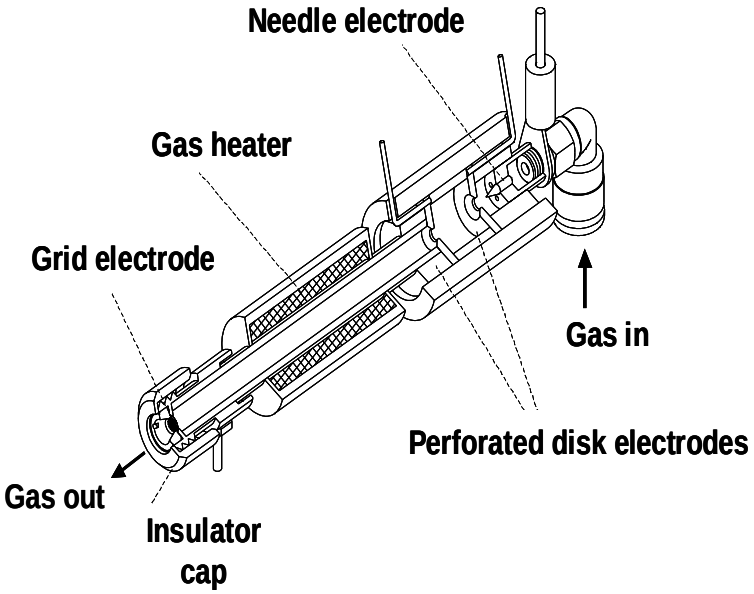

Figure 1-1. Schematic of DART interface (Cody et al., 2005).

The DART-TOF system detects a variety of drugs and their metabolites in urine. Detection limits for analyzing a few microliters of unprocessed urine are in the high part per billion (ng/ml) to low part per million range (µg/ml) (Cody et al., 2005). This range is suitable for detecting overdose levels of common drugs, such as cocaine, morphine, oxycodone, gamma hydroxybutyrate (GHB), ibuprofen, atenolol, and ranitidine. Since early 2005, DART-TOF has been used at the Federal Bureau of Investigation (FBI) Laboratory in Quantico, VA, to rapidly and quantitatively analyze GHB in urine. Preliminary data also exists for detecting drugs in blood samples (see **Figure 1-2**) after protein precipitation.

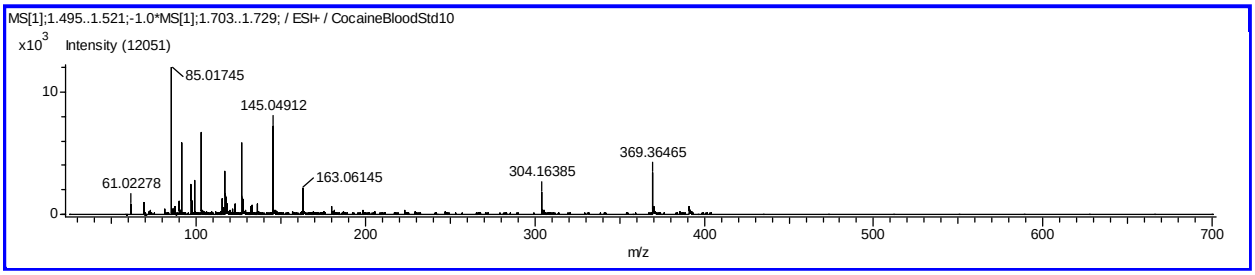

Figure 1-2. 10 µg/mL cocaine detected in 1 mL of blood precipitated with acetonitrile.

Another benefit of the DART-TOF system is that it determines elemental compositions of unknown substances by using exact mass measurements and accurate isotopic abundances. Full-scan, high-resolution data are measured by the TOF mass spectrometer. This can be helpful in identifying unusual or unexpected toxins, whose presence may not be found by conventional single ion monitoring (SIM) or selective reaction monitoring (SRM) experiments. DART-TOF has been used to identify other materials of forensic interest, such as drugs in dose form, inks, dyes, fibers, spermicides, arson accelerants, and explosives. This means the instrument's value is not limited to a single aspect of a death investigation. Furthermore, once methods are established for the postmortem application, they could be easily transferred for use in other forensic analyses conducted in crime laboratories, such as analysis of explosive residues or drug residues.

Currently, very little has been published in the peer reviewed literature about DART-TOF and toxicology or drug chemistries. We have published one paper in *Microgram* in collaboration with the Drug Enforcement Administration (DEA) (Ropero-Miller et al., 2007) comparing the analysis of cocaine to DEA's Cocaine Signature Program analysis. There have been presentations at national meetings by FBI, Harris County Office of the Chief Medical Examiner (OCME), and Virginia Department of Forensic Science (Bennett and Steiner, 2008), but many publications are not yet in print. Presentations at meetings have reported on identifying product adulterants with DART-TOF (Karas et al., 2008) and the successful analysis of controlled substances. Other authors report difficulties in analysis of toxicology samples (Mozayani et al., 2008). Coticone and Roschek (2007) also reported on testing biological samples with DART-TOF and challenges in this analysis.

1.3 Rationale for the Research

The study's purpose was to evaluate the applicability of the direct sample introduction, time of flight mass spectrometer (DART-TOF) technology, to postmortem toxicology analyses. Specifically, this research evaluated the instrument's performance as a screening technology for detecting toxicologically relevant drugs and toxins in postmortem samples and to determine if the instrument could improve the efficiency of biological sample screening. The instrument offers the potential for very rapid sample analysis and has an ability to detect a broad range of compounds with a single analysis. The potential for this combination to work for postmortem samples could greatly enhance the throughput and quality of many laboratories' results. Thus, it is important to verify the potential for the instrument using authentic samples to represent what would be encountered in a postmortem laboratory.

The research was designed to begin evaluating simple matrices and progress to more difficult matrices; each time beginning with no sample preparation and determining if sample preparation (e.g., precipitations, extractions) was required to detect drugs and toxins in a specific matrix. Initial investigations of drugs of interest in postmortem toxicology were prepared in methanol from commercial standards to provide the basis for the evaluation of these drugs in urine. Urine investigation guided the sample preparatory techniques for blood and tissue. In addition to the evaluation of manufactured materials, previously confirmed postmortem case materials were tested (obtained from Offices of the Chief Medical Examiner (OCMEs) in Chapel Hill, NC; Seattle, WA; and Maricopa County, AZ).

Three additional investigations were included in this project beyond the original proposal. The utility of an autosampler for sample introduction was evaluated. Also, the instrument performance was evaluated to determine if components of illicit cocaine could be identified by DART-TOF. A comparison of the same 25 illicit cocaine materials was analyzed by the Cocaine Signature Program, conducted by the DEA special testing laboratory. **Figure 1-3** shows the installation of the instrument with the LEAP technologies autosampler. Finally, two postmortem cases were reanalyzed using five different high-performance liquid chromatography (HPLC)-mass spectral platforms and then compared to the analysis of these same cases on DART-TOF. Again, these additional investigations were ways to improve sample reproducibility of the DART-TOF system and sample throughput for other forensic applications and to evaluate how the instrument could be efficiently used for multiple applications in a laboratory.

Figure 1-3. The DART-TOF system with LEAP autosampler as installed at RTI International.

2. METHODS

The project was divided into four stages.

- Stage I: Analysis of Drugs in Standards
- Stage II: Analysis of Drugs in Urine
- Stage III: Analysis of Drugs in Blood and Tissue
- Stage IV: Additional Study Comparison of Cocaine Analysis with DEA and AFIP

While the evaluations of these components were not separate stages, throughout the project we noted how the instrument performed and how the autosampler provided by LEAP Technologies early in the project was helpful. We also noted how the software performed. We discuss our impressions of these components in Section 3.6 Discussion of the Equipment and Software Performance.

2.1 Stage I: Analysis of Drugs in Standards

Pure drug standards were utilized in this stage. We started with concentrated solutions of model drugs for classes of drugs (e.g., benzodiazepines, atypical antidepressants, stimulants, tricyclic antidepressants, serotonin reuptake inhibitors). Particular attention was paid to drugs for which current screening methods are particularly problematic, such as opioids and benzodiazepines. The total number of compounds examined was 112. **Table 2-1** contains a list of all the compounds assayed.

Table 2-1. List of Compounds Utilized Initially on the DART-TOF

11-hydroxy-Δ9-THC	Dextromethorphan	Nordiazepam
11-nor-Δ9-THC-COOH	Diazepam	Normeperidine
2-hydroxyethylflurazepam	Diphenhydramine	Norpropoxyphene
2-Oxo-3-hydroxy-LAMPA	Ecgonine Methyl Ester	Nortriptyline
2-Oxo-3-hydroxy-LSD	EDDP	Norverapamil
6-MAM	Ephedrine	Olanzapine
7-aminoclonazepam	Fentanyl	Oxazepam
7-aminoflunitrazepam	Fluoxetine	Oxycodone
9-carboxy-11-nor-cannabinol	Flurazepam	Oxymorphone
9-carboxy-11-nor-D9 THC glucuronide	gamma-Butyrolactone	Paroxetine
Δ9-THC/Δ6-THC	GHB	PCP
Acetaminophen	Haloperidol	Pentazocine
alpha-Hydroxy Alprazolam	Heroin	Pentobarbital
alpha-HydroxyTriazolam	Hydrocodone	Phenobarbital
Amitriptyline	Hydromorphone	Phentermine
Amobarbital	Ibuprofen	Phenylpropanolamine
Amphetamine	Imipramine	Phenytoin
Anhydroecgonine	Ketamine	PMA
Anhydroecgonine methyl ester	LAAM	Propoxyphene
Benzoylecgonine	Lorazepam	Psilocybin
Buprenorphine	Lormetazepam	Ranitidine
Buprenorphine Glucuronide	LSD	Scopolamine
Buprenorphine Glucuronolactone	MDEA	Secobarbital

Table 2-1. List of Compounds Utilized Initially on the DART-TOF (continued)

Butalbital	MDMA	Sertraline
Caffeine	Meperidine	Talbutal
Carisopradol	Meprobamate	Temazepam
Chlomipramine	Methadone	Tramadol
Chlorodiazepoxide	Methamphetamine	Trazodone
Chlorpromazine	Midazolam	Triazolam
Citalopram	Morphine	Triethanolamine
Clonazepam	Morphine-3β-glucuronide	Triethylamine
Clonidine	Morphine Glucuronolactone	Tripelannamine
Cocaethylene	Nalbuphine	Venlafaxine
Cocaine	Naproxen	Verapamil
Codeine	N-Ethylamphetamine	Zolpidem
Cotinine	Nicotine	
Desipramine	Norcocaine	
Despropionyl Fentanyl	Norcodeine	

2.1.1 Materials

Drug standards were supplied by Cerilliant (Round Rock, TX). Polyethylene glycol (PEG) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide, KH_2PO_4, and sodium acetate (anhydrous) were also purchased from Sigma. Ethanol was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY); methanol and isopropanol from VWR (West Chester, PA); and acetone, acetonitrile, ether, ethyl acetate, dichloromethane, glacial acetic acid, and butylchloride from Fisher Scientific (Fair Lawn, NJ). All were HPLC grade. Glass melting point tubes were supplied by JEOL USA, Inc.

The DART-TOF (JMS T100LC) was purchased from JEOL USA, Inc. (Peabody, MA).The analyses were conduced in both positive and negative modes of the DART ion source. Instrument settings were as follows:

- Ring lens voltage 5V
- Orifice 1 (O1) voltage 20V
- Orifice 2 (O2) voltage 5V
- Electrode 1 150V
- Electrode 2 350V
- DART temp 300°C
- Detector voltage 2,200V

Predominantly, the M+H or M-H ions, or the parent ion in positive or negative mode respectively, were the desired analyte ions. This optimization produced parent ions for most of the compounds analyzed.

As TOF is a higher mass resolution technique providing accurate mass information rather than relative mass information, the instrument must have a mass calibration. Every analytical run (batch) included a mass calibrant so that each data file could be calibrated against a concurrently analyzed mass reference. Polyethyleneglycol (PEG) was used per manufacturer's recommendation because it has known mass

components spaced regularly across a wide mass range. This provides multiple reference masses in the calibration. **Figure 2-1** provides an example spectrum of PEG. The system was mass-calibrated using PEG before each sample batch. Samples were introduced into the analyzer in one of two ways. The solutions of stock methanolic drug standard were introduced by dipping the glass probe into the solution and passing the probe through the sample gap. Alternatively, the use of an autosampler provided more consistent introduction of samples into the instrument's stream than manual introduction, producing more reproducible spectra. LEAP Technologies (Raleigh, NC) loaned RTI International* (RTI) an autosampler during the project to evaluate its function. **Figure 2-2** shows how the sample can be introduced into the sample gap of the ion source by the LEAP autosampler.

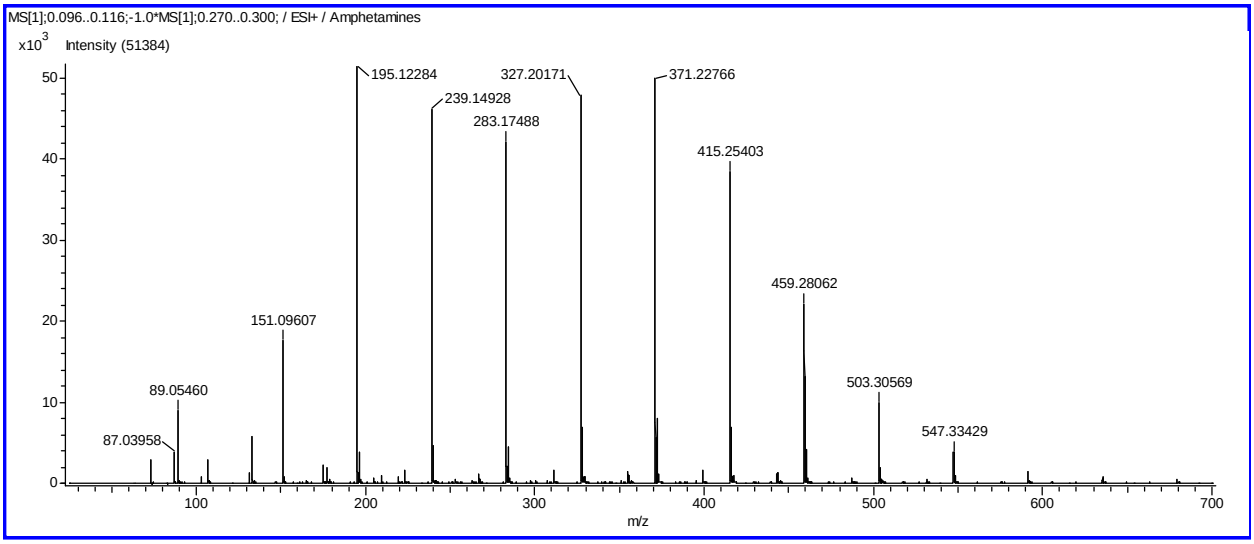

Figure 2-1. Spectrum of polyethylene glycol compound used for mass calibration of DART-TOF.

* RTI International is a trade name of Research Triangle Institute.

Figure 2-2. Sample introduction into the DART-TOF ion source.

2.1.2 Fragmentation Analysis

DART-TOF is a direct introduction ion source mass-spectral method. Because everything is in the source simultaneously, there are no other means of separating compounds for identification other than by accurate mass. While determination of mass to the 1–5 ppm (per mass error) level limits the number of chemical formula that can explain a particular mass, isomers and compounds with the same chemical formula but a different structure cannot be distinguished regardless of resolution and accuracy obtained. Thus, we examined how these compounds might be identified by introducing the sample into the ion source at a greater voltage potential, resulting in unique fragmentation of similar compounds. A list of potential isomer pairs that we identified in our list of compounds is presented in **Table 2-2**. Orifice 1 (O1) voltage was increased step-wise to determine which compounds produced characteristic fragmentations. In the DART-TOF configuration, increasing O1 voltage results in acceleration of ions into the instrument and subsequent fragmentation of compounds.

Table 2-2 lists isomer pairs and compounds of very close masses. Of the compounds tested, there were nine pairs of exact isomers. Ten other compounds were close enough in theoretical monoisotopic mass to potentially be problematic and were evaluated by this procedure as well.

Table 2-2. Isomer and Near-isomer Pairs of Compounds Evaluated by DART-TOF at RTI

Drug	M+H	Near Isomers	M+H
Pentobarbital/Amobarbital	227.138	Phentermine/triethanolamine	150.120/150.105
Codeine/Hydrocodone	300.159	Methamphetamine/triethanolamine	150.120/150.105
2-Oxo-3-hydroxy LSD/3-Oxo-3-hydroxy LAMPA	356.196	Amitriptyline/venlafaxine	278.183/278.204
Morphine/Hydromorphone/Norcodeine	286.143	Tramadol/nortriptyline	264.188/264.167
Benzoylecgonine/Norcocaine	290.138	Ranatidine/Clomipramine	315.141/315.154
PMA/Ephedrine	166.122	Diphenhydramine/Tripelennamine	255.162/255.173
Cocaine/Scopolamine	304.154		
Talbutal/Butalbutal	225.123		
Phentermine/methamphetamine	150.120		
∆9-THC/∆6-THC	315.224		
Imiprimine/Despropyl Fentanyl	281.193		

2.2 Stage II: Analysis of Drugs in Urine

After optimization of the instrument for the detection of pure solutions of standards in Stage I, we evaluated the instrument's performance with compounds spiked into a urine matrix. The drugs chosen were determined in part based on the drug content of routine postmortem cases.

Limits of detection were evaluated by diluting solutions until the peak of interest was no longer identifiable either by deviating more than 8–10 mmu from the theoretical monoisotopic mass or the peak was not discernible from the background. This provided an assessment of the technology's applicability to other analytes in the same structural class. Positive ionization was used for this investigation.

Detection of target compounds in neat urine (i.e., no extraction or other sample preparation treatments) was evaluated. Based upon these results, the use of a sample preparation was evaluated. This involved the liquid-liquid extraction of the urine (2 mL) into 3:1 butylchloride/ether (2 mL). The goal was to achieve screening results with minimal sample preparation. A requirement for extensive sample preparation (e.g., solid-phase extractions and/or back extractions and significant concentration or derivatization steps) would significantly limit the instrument's application in postmortem toxicology work. Stock drug standards (1 mg/mL) were diluted into the appropriate volume of blank urine to prepare 100 µg/mL of working standard solutions. The working solutions were serial diluted until the limit of detection (LOD) was reached. We also evaluated the instrument's performance with 10 postmortem urine samples from archived cases.

The DART-TOF parameters were the same as reported in the previous section.

We compared our findings to the original reported findings of the individual cases. Only analytical results were obtained from the postmortem toxicology laboratory for comparison. Case-identified information for the samples was not obtained.

2.3 Stage III: Analysis of Drugs in Blood and Tissue

One of the most challenging components of forensic toxicology is the analysis of blood and tissue samples. The extent of this instrument evaluation stage was determined largely by the number and type of samples we obtained.

Table 2-3 gives a distribution of the number and types of cases analyzed. Correlation of screening results obtained with the DART-TOF system to those obtained in the original analyses of these cases was also case dependent, given that the original analyses of these types of samples were likely problematic.

Table 2-3. Number of Cases Analyzed of Each Tissue Type

Specimen	Cases Analyzed
Blood	28
Liver	11
Kidney	2
Brain	4

Typically, matrices such as these were difficult to analyze originally, and the interpretation of correlation between the DART-TOF system and the original analysis was cautious. For example, original analyses may not have analyzed for all of the drugs we investigated based on an individual laboratory's decision process of testing assignments. The list of drugs (**Table 2-4**) was determined in part by the drugs present in the postmortem cases available. A total of 50 drugs were previously detected in the original analysis and screened for using the DART-TOF system.

Blood fortified with drugs of interest were evaluated first. Specifically, hemolyzed blood was used for constructed test samples because it is the most common postmortem blood sample. In these studies, drugs were spiked into human blank blood supplied by North Carolina's OCME. The blood was hemolyzed using sodium fluoride and sodium chloride (a common preservative found in "grey top" blood collection tubes).

Early evidence indicated that blood sample analysis by DART-TOF required precipitation of proteins prior to sample introduction to the ion source. We examined a sample precipitation and concentration method using acetonitrile. The objective was to determine if an expedient precipitation and concentration would result in improved detection of compounds. We then evaluated the instrument's performance with postmortem blood samples and tissues from archived cases in a manner similar to that used for urine.

We also analyzed several cases after solid phase extraction (SPE). In a further study, results from one cocaine case and one methadone case prepared by SPE and analyzed by DART-TOF were compared to several other MS technologies (Stage V). These samples were analyzed on a consistent LC system with five separate MS and tandem mass-spectral (MS/MS) platforms for detection.

Table 2-4. Drugs in Tissue and Blood that Were Previously Reported in the Cases Analyzed

Acetaminophen	Dextromethorphan	Morphine
Alprazolam	Diazepam	Nicotine
Amphetamine	Diphenhydramine	Nortriptyline
Atropine	EME	Olanzapine
Benzoylecgonine	Fluoxetine	Oxcarbazepine
Caffeine	Fluvoxamine	Oxycodone
Carbamazepine	Gabapentin	Oxymorphone
Carisoprodol	Hydrocodone	Paroxetine
Chlopheniramine	Ibuprofen	PCP
Citalopram	Levorphanol	Promethazine
Clonazepam	MDMA	Propoxyphene
Cocaine	Methamphetamine	Quetiapine
Codeine	Methadone	Temazepam
Cotinine	Methylphenidate	THC
Cyclobenzaprine	Meprobamate	Topiramate
Desmethylcitalopram	Metoprolol	Trazodone
Desmethyldoxepin	Mirtazapine	

Tissue samples (i.e., brain, liver) were analyzed by freshly homogenizing the samples in water. Five grams of tissue was homogenized in 15 mL of water. A Kinematica Polytron (Bohemia, NY) homogenizer was used to homogenize tissue samples for 10 minutes on a setting of 6. The homogenate was introduced directly into the instrument's stream, and the homogenate was precipitated and concentrated by the same method as described below prior to analysis.

2.3.1 Liquid/Liquid Precipitation and Concentration Method

After blood and tissue samples were analyzed neat (i.e., direct introduction of the sample into the DART source), a liquid precipitation and concentration step was evaluated both for sample preparation and for its potential to yield improved results. In part, the concept of the treatment was to help precipitate proteins, provide a cleaner sample for analysis, and concentrate the sample. Two milliliters of acetonitrile (ACN) was added to 1 mL of each specimen to precipitate proteins. The mixture was shaken, vortexed, and spun in a centrifuge at 3,000 rpm for 7 minutes. Approximately 100 μL of the ACN layer was saved for DART-TOF analyses. As a sample concentration method, the remainder was poured off and dried down at 45^{o}C under nitrogen, reconstituted in 100 μL of acetonitrile, and analyzed. All DART-TOF parameters were set as previously stated. As determined in Stage 1, most drugs of interest produced an M+H ion, so samples were run in positive mode.

2.3.2 Solid Phase Extraction Method

In addition to the rapid precipitation method, we evaluated a more extensive sample preparation using SPE to evaluate the data obtained after a more complete technique was used to isolate the drug from biological matrices. SPE is used with most traditional chromatographic methods. For this purpose, cocaine and opioid analytes were extracted by the following methods:

Cocaine Extraction: 2 mL of specimen was diluted 1:3 in phosphate buffer (pH 6). The sample was then vortexed and centrifuged at 3,000 rpm for 7 minutes. The sample was transferred into CLIN II columns (SPEWare, Santa Clara, CA) and allowed to flow through at a flow rate of 10–20 mL/min. The columns

were washed with deionized water (1 mL), 100 mM HCl (1 mL) and dried at 25 psi for 2 minutes. The columns were further washed with methanol (1 mL) and ethyl acetate (1 mL) and dried a second time. Samples were eluted at 1 mL/min with an 80/20/2 dichloromethane/IPA/ammonium hydroxide (2 mL) solution, evaporated to dryness at 45°C under nitrogen and reconstituted in 100 µL ACN.

Opiate Extraction: 2 mL of specimen was diluted 1:3 in 1.0 M acetate buffer (pH 5). The sample was then vortexed and centrifuged at 3,000 rpm for 7 minutes. The sample was transferred into CLIN II columns and allowed to flow through at a flow rate of 10–20 mL/minute. The columns were washed with deionized water (1 mL), 100 mM acetate buffer, methanol (1mL), ethyl acetate (1 mL) and dried down at 35 psi for 2 minutes. The samples were evaporated to dryness at 45°C and reconstituted in 100 µL ACN.

2.3.3 LC/MS and MS/MS Analyses (additional study as a modification from original proposal)

Because older postmortem cases were used in this study, one concern was that failure to detect previously reported compounds may have resulted from the degradation of samples and not from limitations of DART-TOF instrumentation. Additionally, a direct comparison of more traditional LC/MS and liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis of the same case materials as analyzed by DART-TOF provided a useful opportunity to evaluate advantages of the DART-TOF system. In a cooperative study with Agilent Technologies and The University of Miami, two postmortem cases were analyzed (one with cocaine and one with methadone) by DART-TOF and compared to analysis with a common LC method and various MS and MS/MS platforms. MS technologies included single quadrupole MS, triple quadrupole MS/MS, ion trap MS, time of flight MS and Q-TOF MS. **Table 2-5** provides the original confirmation results for these two cases and case information.

Table 2-5. Previously Reported (Original Laboratory Analysis) Postmortem Case Results for Comparison of DART-TOF to Other Mass Spectral Technologies

Case 1	**mg/L**
Cocaine	0.03
EME	0.22
BE	1.76
Oxycodone	0.08
Propoxyphene	0.45
Norpropoxyphene	0.91
Alprazolam	Positive
Case 2	**mg/L**
Methadone	1.1
EDDP	Positive
Sertraline	0.14
Desmesthylsertraline	0.76
Doxepin	0.74
Desmethyldoxipin	3.78

2.3.4 LC Conditions—Used for All MS Platforms

A 1200 Series RRLC system with binary pump SL (Agilent Technologies) was equipped with a Zorbax Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm (Agilent PN: 959764-902) column. Column temperature was maintained at 50° C. Solvents used were as follows: A: Water + 5mM Ammonium formate + 0.05 %

Formic Acid, B: Acetonitrile + 0.05 % Formic Acid. Flow rate was 250 µL/min. Method gradient was as follows:

Time (min)	% Solvent B
1.0	5
6.0	40
8.0	95

The LC method stop time was 10 min with an additional 2 min post time for instrument equilabration. Injection volume for final analysis was 5 µL (SQ, QQQ & Trap), 2 µL (TOF), 0.1 µL (Q-TOF). Analysis by the Q-TOF and TOF required less volume because the systems demonstrated too much signal at 5 µL.

LC/MS (single Quad) analysis used an Agilent 6140 Mass Spectrometer. For all sources, the ion source parameters were similar. The source was electrospray in positive mode. Other LC/MS parameters included the following: capillary 3,000V; N_2 drying gas 10 L/min; nebulizer pressure 30 psi; gas temperature 350°C. **Table 2-6** contains the ions monitored by LC/MS, fragmentor voltage, and dwell time.

Table 2-6. LC/MS Parameters

Time (min)	Compound	SIM ion	Fragmentor (V)	Dwell(msec)
0.0	Cocaine	304.1	125	75
	Cocaine-D3	307.1		
	Benzoylecgonine	290.1		
	Benzoylecgonine-D3	293.1		
	Cocaethylene	318.1		
	Cocaethylene-D3	321.1		
7.0	Methadone	310.2	125	235
	Methadone-D3	313.2	125	235

LC/MS/MS (Triple Quad; QQQ) analysis used an Agilent 6140 QQQ Mass Spectrometer. Similar parameters reported for LC/MS were also used for this system. **Table 2-7** contains additional LC/MS/MS settings, including ions monitored, multiple reaction monitoring (MRM), fragmentor voltage, collision energy, and dwell time.

Table 2-7. Parameters Used in the Triple Quad Analysis Utilizing the Agilent 6410 QQQ Mass Spectrometer

Time (min)	Compound	MRM	Fragmentor (V)	Collision Energy (V)	Dwell(msec)
0.0	Cocaine	304.1 > 182 [82]	130 [130]	15 [30]	40
	Cocaine-D3	307.1 > 185	130	15	
	Benzoylecgonine	290.1 > 168 [105]	110 [110]	15 [30]	
	Benzoylecgonine-D3	293.1 > 171	110	15	
	Cocaethylene	318.1 > 196 [82]	130 [130]	15 [30]	
	Cocaethylene-D3	321.1 > 191	130	15	
8.0	Methadone	310.2 > 265.1 [105]	110 [110]	15 [25]	75
	Methadone-D3	313.2 > 268	110	15	

The ion trap platform used was the Agilent 6330 Ion Trap Mass Spectrometer. Parameters included the following: targeted screening—with AutoMS providing MS^3 information including a library of expected compounds. Automated library searching/reporting was used.

Analysis of the TOF platform utilized an Agilent 6210 accurate-mass TOF Mass Spectrometer. Analysis scanned *m/z* 100–1000, 10,000 transients/scan. Mass calibration was continuously updated by automatic reference masses using *m/z* 121 and 922. Theoretical monoisotopic masses (M+H) used are in **Table 2-8.**

Table 2-8. Theoretical Monoisotopic Masses Used for TOF Platforms (TOF and Q-TOF)

Analyte	Chemical Formula	Theoretical Monoisotopic Mass	M+H
Benzoylecgonine	$C_{16}H_{19}NO_4$	Mr 289.1314	290.1387
Cocaine	$C_{17}H_{21}NO_4$	Mr 303.1571	304.1543
Cocaethylene	$C_{18}H_{23}NO_4$	Mr 317.1627	318.1700
Methadone	$C_{21}H_{27}NO$	Mr 309.2093	310.2165
Alprazolam	$C_{17}H_{13}N_4Cl$	Mr 308.0829	309.0902
Nordiazepam	$C_{15}H_{12}N_2OCl$	Mr 270.0560	271.0633
Diazepam	$C_{16}H_{14}N_2OCl$	Mr 284.0716	285.0789

2.4 Stage IV: Additional Study Comparison of Cocaine Analysis with DEA and AFIP (additional study as a modification to the original proposal)

RTI was also conducting research on NIJ grant no. 2006-DN-BX-K019 during the time period of this project. In doing this, we were working with the Drug Enforcement Administration (DEA) and the Armed Forces Institute of Pathology (AFIP) to identify components of 25 illicit cocaine samples. RTI utilized this opportunity to compare the results of these cocaine samples to DART-TOF analysis. In addition to postmortem analysis, DART-TOF could be very advantageous for this type of analysis. While cocaine signature analyses have become routine in many forensic laboratories, these laboratories could benefit from a rapid screening method to identify controlled substances (Ehleringer et al., 2000). A procedure with minimal to no sample preparation could be used to complement traditional methods to identify constituents of cocaine. Not only could DART-TOF potentially identify cocaine analytes but adulterants

(e.g., sugars, prescription and illicit drugs) and illicit manufacturing solvents as well. Determination of adulterants can help law enforcement trace manufacturing trends.

Twenty-five illicit cocaine hydrochloride samples were obtained with permission from the National Institute of Drug Abuse's Division of Neuroscience & Behavioral Research (Bethesda, MD).

2.4.1 DEA Cocaine Signature Analyses by Gas Chromatography/Mass Spectrometry (GC/MS)

Cocaine signature analyses consisted of GC-MS as reported by Casale and colleagues and are briefly described herein (Ehleringer et al., 2000; Casale and Waggoner, 1991; Casale and Moore, 1994; Morello and Meyers, 1995; Moore et al., 1996). Analyses were performed using Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34–700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm internal diameter fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C; no hold, program rate, 6°C /min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The auxiliary transfer line to the MSD was operated at 280°C.

2.4.2 AFIP Analysis of Illicit Cocaine Samples by GC/MS

An SPE extraction to isolate COC eluting with CH_2Cl_2:isopropanol:aqueous ammonia (8:2:0.2) was employed. Samples reconstituted in acetone (100 uL) were analyzed by GC/MS. GC/MS parameters included the following: GC oven: 100°C (1 min) to 260°C at 30°C /min and then to 280°C (1 min) at 10°C /min; injection: 120°C; auxiliary 280°C; MS ions: 303, 272, and 182 for cocaine; 306 and 185 for d_3-cocaine.

A separate SPE extraction [CH_2Cl_2:isopropanol:aqueous ammonia (8:2:0.2)] was used to isolate CE, anhydroecgonine methyl ester (AEME), and norcocaine (NCOC). The residues after extraction were dissolved in acetone (120 μL) and divided in two equal portions. One portion was tested directly for CE and AEME by GC/MS with the following parameters: GC oven: 100°C (1 min) to 270°C (1 min) at 30°C/min; injection: 120°C; auxiliary 280°C MS ions: 317, 272, and 196 for CE; 325 and 204 for d_8-CE; 181, 166, and 152 for AEME; 184 and 155 for d_3-AEME.

The second portion of the extract was evaporated to dryness and derivatized with 50 μL of pentafluoropropionic anhydride (50 ul, 70°C/30 min). The samples were again evaporated to dryness, reconstituted in 40 ul of dry acetonitrile, and tested by GC/MS with the following parameters: GC oven: 150°C (1 min) to 280°C (2 min) at 35°C/min; injection: 170°C; auxiliary 280°C; MS ions: 313, 194, and 166 for NCOC; 316 and 197 for d_3-NCOC.

2.4.3 AccuTOF-DART Analysis

The DART-TOF parameters were set as previously mentioned. All samples were introduced into the ion source manually by hand, dipping a glass probe into the sample and passing this through the stream. When available, the monoisotopic M+H values of the cocaine analytes were verified using certified drug standard solutions.

3. RESULTS

3.1 Stage I: Findings from Analysis of Standard Materials

After both positive and negative ionization analysis of the 112 standard compounds, M+H and M-H ions were not seen for four compounds. **Table 3-1** lists all of the drugs tested and the expected and measured masses.

Table 3-1. Compounds, M+H Monoisotopic Masses, and M-H Observations of DART-TOF Analysis of Standard Materials

Compound	Theoretical Monoisotopic Mass + H	Measured Mass + H	M-H Observed
Acetaminophen	152.0708	152.071	
alpha-Hydroxy alprazolam	325.0848	325.085	
Amitriptyline	278.1908	278.193	
Amobarbital	227.1388	227.137	
Amphetamine	136.1118	136.112	
Anhydroecgonine	168.1018	168.102	
Anhydroecgonine methyl ester	182.1178	182.115	
Benzoylecgonine	290.1388	290.136	
Buprenorphine	468.3108	468.309	Yes
Butalbital	225.1238	225.125	Yes
Caffeine	195.0878	195.086	
Carisopradol	261.1808	261.180	Yes
Chlorodiazepoxide	300.0898	300.087	Yes
Clorpromazine	319.1028	319.102	
Chlomipramine	315.1618	315.161	
Citalopram	325.1708	325.170	
Clonazepam	316.0488	316.048	Yes
7-Aminoclonazepam	286.0738	286.075	Yes
Clonidine	230.0248	230.024	Yes
Cocaethylene	318.1698	318.167	
Cocaine	304.1548	304.153	
Codeine	300.1598	300.157	Yes
Cotinine	177.1018	177.103	
Despropionyl Fentanyl	281.2008	281.199	
Desipramine	267.1858	267.185	
Dextromethorphan	272.2008	272.202	
Diazepam	285.0788	285.078	Yes
Diphenhydramine	256.1698	256.169	
Ecgonine Methyl Ester	200.1278	200.127	
EDDP	278.1908	278.190	Yes
Ephedrine	166.1228	166.122	
N-Ethylamphetamine	164.1438	164.145	
Fentanyl	337.2278	337.227	
7-Aminoflunitrazepam	284.1198	284.121	Yes
Fluoxetine	310.1418	310.143	
Flurazepam	388.1588	388.160	Yes
2-Hydroxyethylflurazepam	333.0798	333.079	Yes
gamma-butyrolactone	87.0438	87.044	Yes

Compound	Theoretical Monoisotopic Mass + H	Measured Mass + H	M-H Observed
gamma hydroxybutyrate	105.0548	105.054	Yes
Haloperidol	376.1478	376.147	Yes
Heroin	370.1648	370.164	
Hydrocodone	300.1598	300.157	
Hydromorphone	286.1438	286.141	Yes
Ibuprofen	207.1378	207.137	Yes
Imipramine	281.2008	281.199	
Ketamine	238.0998	238.101	
LAAM	354.2428	354.243	
2-Oxo-3-hydroxy-LAMPA	356.1968	356.194	Yes
Lorazepam	321.0188	321.019	Yes
Lormetazepam	335.0348	335.034	Yes
LSD	324.2068	324.206	Yes
2-Oxo-3-hydroxy-LSD	356.1968	356.196	Yes
6-AM	328.1548	328.152	Yes
MDEA	208.1328	208.135	
MDMA	194.1178	194.117	
Meperidine	248.1648	248.163	
Meprobamate	219.1338	219.132	Yes
Methadone	310.2168	310.214	Yes
Methamphetamine	150.1278	150.129	
Midazolam	326.0858	326.084	Yes
Morphine	286.1438	286.141	Yes
Nalbuphine	358.2018	358.203	Yes
Naproxen	231.1018	231.101	
Nicotine	163.1228	163.123	
Normeperidine	234.1488	234.150	
Norcocaine	290.1388	290.140	
Norcodeine	286.1438	286.143	
Nordiazepam	271.0628	271.062	Yes
Norpropoxyphene	326.2118	326.214	
Nortriptyline	264.1748	264.173	
Norverapamil	441.2828	441.283	
Olanzapine	313.1478	313.147	Yes
Oxazepam	287.0578	287.056	Yes
Oxycodone	316.1548	316.154	Yes
Oxymorphone	302.1388	302.139	Yes
Paroxetine	330.1498	330.148	
PCP	244.2058	244.206	
Pentobartital	227.1388	227.138	Yes
Pentazocine	286.2168	286.217	Yes
Phenobarbital	233.0918	233.094	Yes
Phentermine	150.1278	150.129	
Phenylpropanolamine	152.1068	152.107	
Phenytoin	253.0968	253.097	
PMA	166.1228	166.123	
Propoxyphene	340.2268	340.224	Yes

Compound	Theoretical Monoisotopic Mass + H	Measured Mass + H	M-H Observed
Ranitidine	315.1488	315.151	Yes
Scopolamine	304.1548	304.152	
Secobarbital	239.1388	239.136	Yes
Sertraline	306.0808	306.080	
Talbutal	225.1238	225.126	Yes
Temazepam	301.0738	301.073	Yes
11-nor-Δ9-THC-COOH	345.2058	345.207	Yes
Δ9-THC/Δ6-THC	315.2318	315.231	Yes
11-hydroxy-Δ9-THC	331.2268	331.230	Yes
Tramadol	264.1958	264.194	
Trazodone	372.1588	372.158	
Triazolam	343.0508	343.048	Yes
alpha-Hydroxy triazolam	359.0458	359.046	Yes
Triethanolamine	150.1128	150.112	Yes
Tripelennamine	256.175	256.173	
Triethylamine	102.1278	102.127	
Venlafaxine	278.2118	278.212	
Verapamil	455.2908	455.290	
Zolpidem	308.1758	308.171	Yes
Morphine 3β glucuronide	462.1758	462.181	
Morphine	286.1438	286.141	Yes
Glucuronolactone	177.0398	177.041	
Buprenorphine glucuronide*	644.3428	ND	
Buprenorphine	468.3108	468.322	
Glucuronolactone	177.0398	177.050	
9-carboxy-11-nor-D9 THC glucuronide	521.2378	ND	
9-carboxy-11-nor-cannabinol	341.2058	ND	
Psilocybin	284.0998	ND	

ND = none detected

* Buprenorphine glucuronide was not detected but components, buprenorphine and glucuronolactone were detected

Four compounds were not detected from the original list of compounds. These four were buprenorphine-glucuronide, 9-carboxy-11-nor-tetrahydrocannabinol, 9-carboxy-11-nor-D9 THC glucuronide, and psilocybin. While buprenorphine-glucuronide was not detected, the buprenorphine and glucuronolactone could be detected. All other compounds tested produced an expected M+H ion. Sixty compounds tested did not produce an expected M-H ion. As well as producing the M+H ion, many compounds also produced a dimer +H of the compound in positive mode. Dimer ions could assist in the determination of an unknown compound. Mass accuracy was very good for most compounds; however, mass accuracy was high for a few compounds for undetermined reasons. Mass accuracy was affected by sample introduction dynamics and can pose a challenge.

Spectra of compounds are presented in **Appendix A**. At the start of each appendix there is a table of contents providing the page number of the spectra. Spectra are arranged into drug classes. Spectra from both the positive mode analysis and negative mode analysis of all drugs identified are included in this appendix. For each drug class, a total ion chromatograph (TIC) is provided to indicate which drugs were analyzed together in a given analytical run. These are provided for completeness of the information but do not have any bearing on chromatographic separation because DART-TOF is not a chromatographic

method. Rather, these TICs provide an indication of the variability of the relative response of various compounds measured as the "intensity" on the *y*-axis. The scale of intensity was not standardized among TICs. Each of the peaks in the TIC represent the introduction of the melting point probe with sample into the instrument's stream.

In each drug spectra, the theoretical monoisotopic mass of the M+H ion is labeled. As can be seen in many spectra, the negative mode analysis resulted in a mode complex spectra that often did not provide easily explained fragments or adducts. Many positive mode spectra, while containing the expected M+H ion, often also contain other fragments and adducts. While in isolation, these may be useful in the identification of compounds; in the mixed environment of an unknown biological specimen, these masses would be of little use for identification and may not even be consistently formed in the analysis of a biological sample. Thus, the parent M+H ion was the focus of analysis.

During the analysis, 14 potential isomer or near-isomer pairs were identified. These pairs of compounds were exact isomers or had monoisotopic masses similar enough to be difficult to resolve due to isotopic contributions. These compounds were analyzed in M+H mode using different parameters to fragment each compound for identification and examination if characteristic fragmentation could be obtained. The analysis of isomer pairs, such as cocaine and scopolamine was conducted in positive analysis mode by raising the voltage on the entrance orifice cone (O1) from 5V to 90V in order to produce fragments of each compound. **Figure 3-1** provides a comparison of cocaine and scopolamine with a change in the voltage on O1. Distinct and reproducible fragments were apparent. In contrast, morphine and norcodeine (**Figure 3-2**) did not produce consistent fragments. With this exception, all other isomer pairs were distinguishable by unique ions from fragmentation.

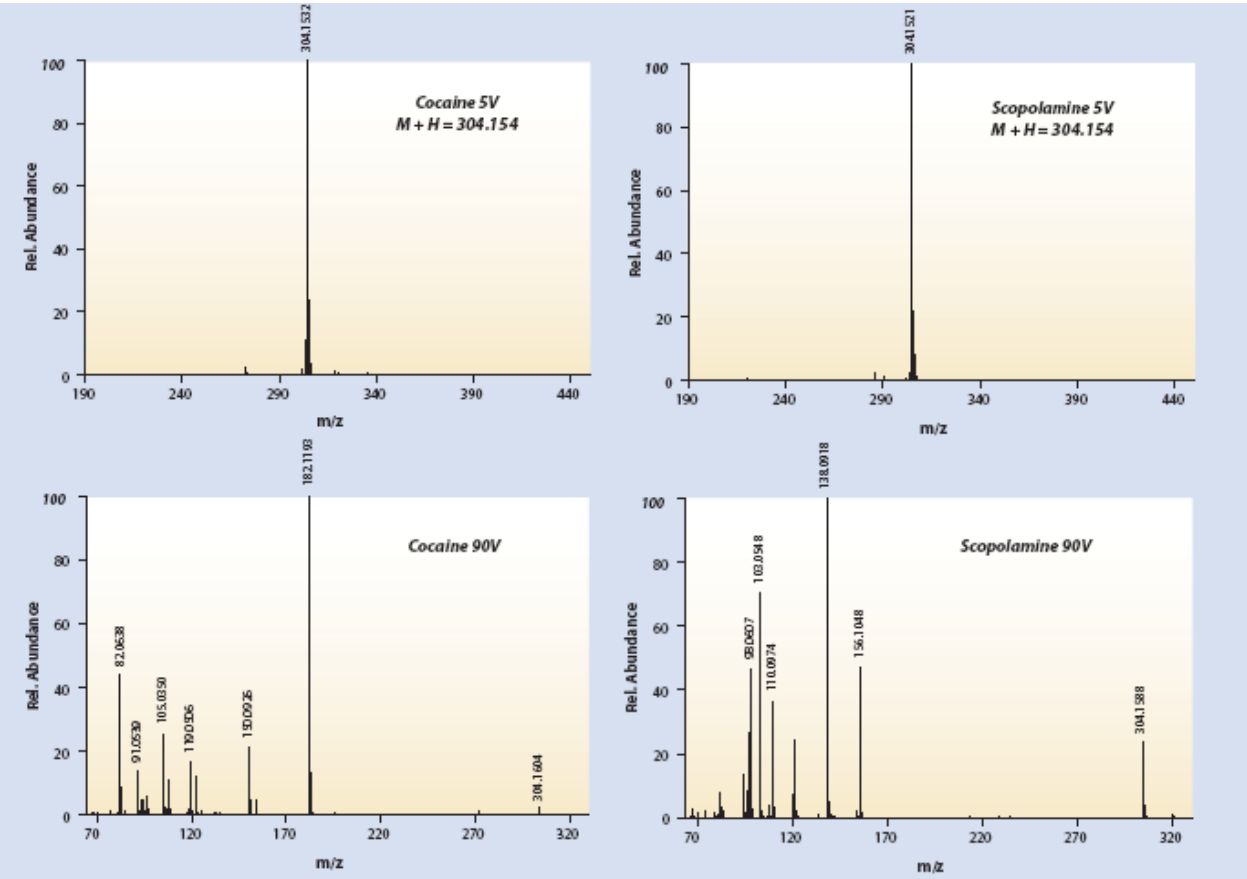

Figure 3-1. A comparison of the fragmentation of cocaine and scopolamine by changing the Orifice 1 voltage. Note the different fragment masses produced.

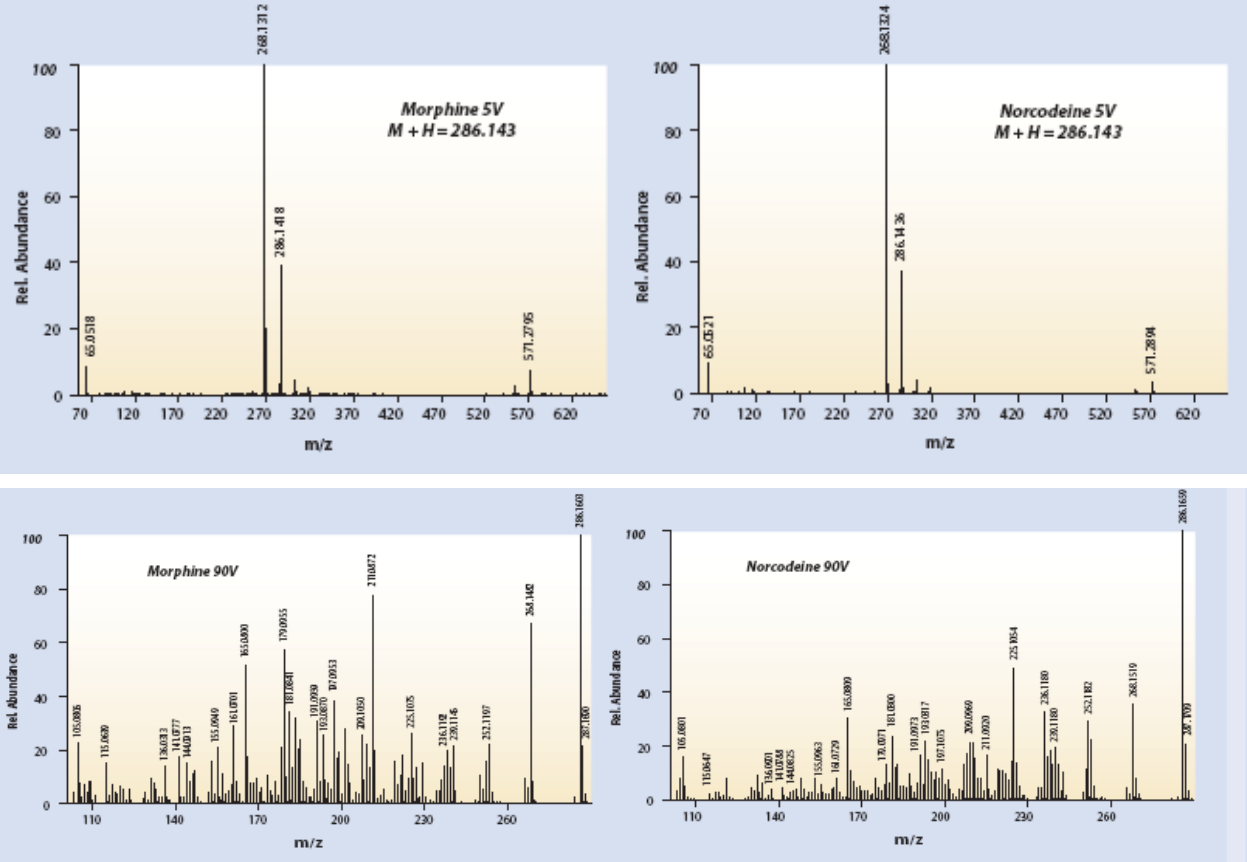

Figure 3-2. A comparison of morphine and norcodeine fragments produced by different Orifice 1 voltages. Note the lack of any consistent or notable fragment masses.

As can be seen in Figure 3-1, cocaine can be distinguished from scopolamine by utilizing different O1 voltages, but in Figure 3-2 morphine and norcodeine cannot be resolved by fragmentation. Additionally, in mixed unknown samples, the resolution of isomers by fragmentation is problematic due to the potential for many multiple masses to be present. **Table 3-2** provides a summary of the unique fragmentation observed for all of the isomer pairs or near isomer pairs analyzed. Appendix A (A-10) contains 90V spectra for all of these compounds.

Table 3-2. Summary of the Unique Fragmentation

Drug	M+H	Unique Fragmen-tation	Near Isomers	M+H	Unique Fragmen-tation
Pentobarbital/Amobarbital	227.138	Yes	Phentermine/ triethanolamine	150.120/150.105	Yes
Codeine/Hydrocodone	300.159	Yes	Methamphetamine/ triethanolamine	150.120/150.105	Yes
2-Oxo-3-hydroxy LSD/3-Oxo-3-hydroxy LAMPA	356.196	Yes	Amitriptyline/ venlafaxine	278.183/278.204	Yes
Morphine/Hydromorphone/ Norcodeine	286.143	No	Tramadol/nortriptyline	264.188/264.167	Yes
Benzoylecgonine/ Norcocaine	290.138	Yes	Ranatidine/ Clomipramine	315.141/315.154	Yes
PMA/Ephedrine	166.122	Yes			
Cocaine/Scopolamine	304.154	Yes			
Talbutal/Butalbutal	225.123	Yes			
Phentermine/ methamphetamine	150.120	Yes			

The DART-TOF successfully identified 108 compounds by positive ionization commonly analyzed in postmortem forensic testing from manufactured standard solutions. By negative ionization, 50 compounds (49%) were successfully identified. The data obtained from each analysis, including isomer fragmentations, are stored within the instrument's library search program for future comparison. The creation of these libraries enhances the DART-TOF by providing fast and reliable data analysis. Libraries generated in this manner could potentially be shared with other laboratories to promote standardization of future methods. Based on the results of these standards, the DART-TOF was operated in positive mode for subsequent sections because most of the drugs examined produced expected M+H ions. Negative mode may be useful in analysis of some compounds, but of the compounds tested, negative ionization mode did not produce particularly useful data.

3.2 Stage II: Analysis of Drugs in Urine

Upon completion of analysis of drug standards in methanol, we proceeded to the analysis of compounds in urine. **Appendix B, Drugs in Urine,** provides a more complete collection of spectra (e.g., compounds from each drug class and compounds at different concentrations). While mass peaks for various compounds are detectable at the single µg/mL concentrations, the size of the peak is such that realistic detection of the compound would be problematic in an unknown sample. In this appendix, representative drugs from each class are provided with spectra at decreasing concentrations in urine. All are spectra obtained from neat urine directly introduced into the instrument's stream. **Figure 3-3** provides a spectrum of blank human urine. Notably common urine constituents, urea (61.046), creatinine (114.071), and a dimer of creatinine (227.135) are readily visible in most urine specimens. **Table 3-3** shows the LOD for 28 urine spiked standards.

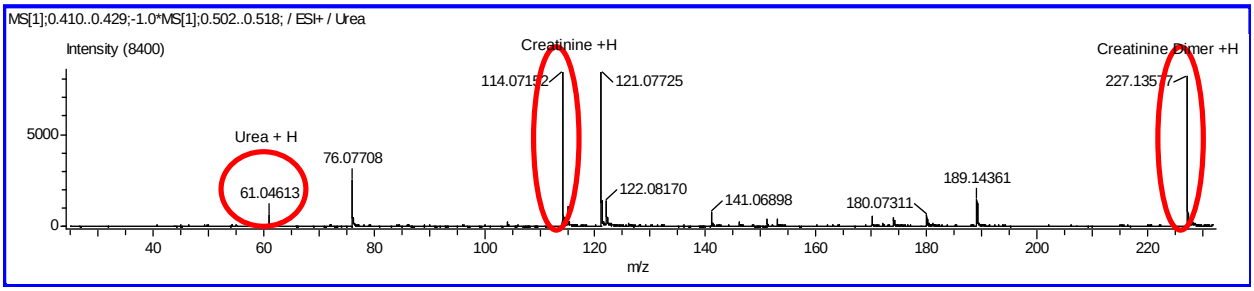

Figure 3-3. Spectra of blank human urine with presence of urea+H (61.046), creatinine+H (114.071), and creatinine dimer+H (227.135).

Table 3-3. The Limit of Detection for 28 Spiked Urine Standards

Drug	LOD (µg/mL)	Drug	LOD (µg/mL)
Amitriptyline	0.78	LSD	3.1
Amobarbital	50	MDEA	12.5
Amphetamine	1.5	Methadone	1.5
Benzoylecgonine	12.5	Methamphetamine	6.2
Caffeine	15	Morphine	3.1
Cocaethylene	12.5	Nicotine	1.5
Cocaine	1.2	Oxycodone	3.1
Codeine	12.5	Oxazepam	25
Dextromethorphan	3.1	PCP	0.78
Diphenhydramine	1.5	Pentobarbital	6.2
Diazepam	3.1	Phenylpropanolamine	6.2
Ecgonine Methyl Ester	1.5	Propoxyphene	0.78
Fentanyl	0.31	Δ9-THC	3.1
Fluoxetine	6.2	Trazodone	50
Heroin	25	Triazolam	3.1
Hydrocodone	3.1		

Trazodone and amobarbital had the highest LOD at 50 µg/mL, while fentanyl had the lowest at 0.31 µg/mL. Generally, the LODs are high for most compounds for postmortem testing; however, some are close to relevant concentrations. Mass accuracy for the analytes was better than 8 mmu. As the concentration begins to decrease, the mass peak shape begins to affect the mass accuracy. Sensitivity was often limited by mass accuracy. However, in the analysis of real samples, very small peaks, even with accurate masses, would be very difficult to reliably identify from the background. Detection of compounds would be additionally challenging in more complex unknown samples. **Figure 3-4** shows a difference in the intensity of the response in example spectra of methamphetamine and triazolam at the same concentration (100 µg/mL). This appears to have been related to creatinine in the sample.

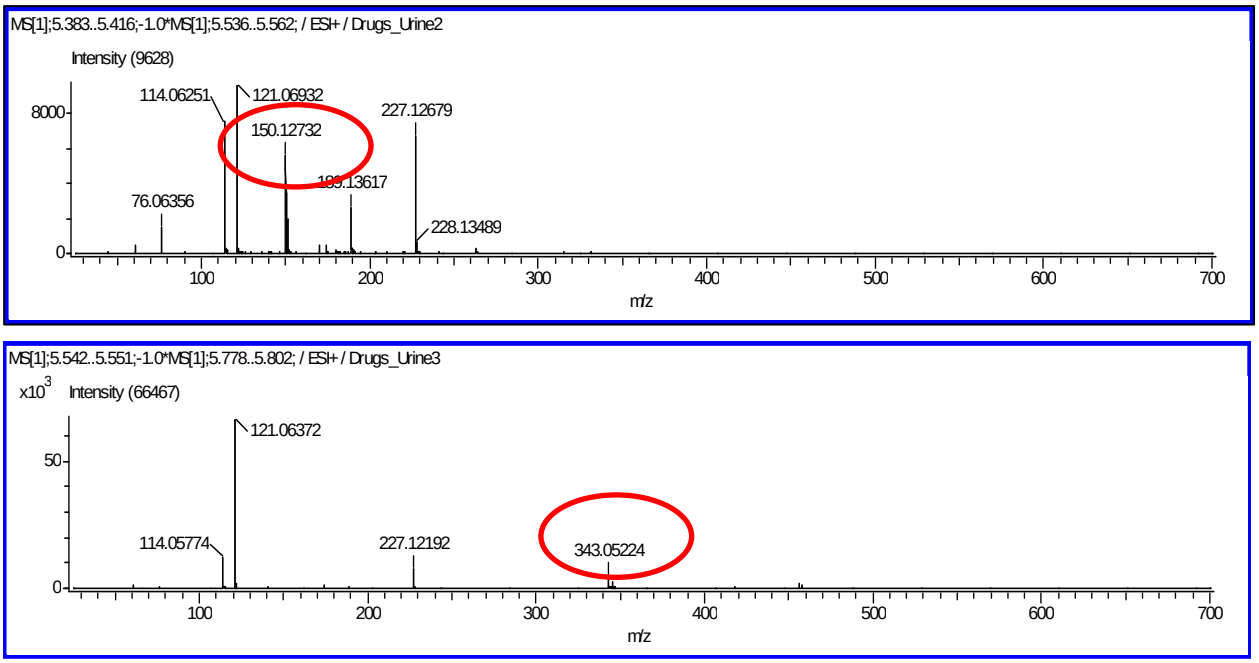

Figure 3-4. DART-TOF spectrum of methamphetamine (150.127) and triazolam (343.052) at 100 μg/mL in urine.

While all of these examples show $(M+H)^+$ values ≤0.002 *m/z* different from their theoretical monoisotopic masses, the intensity of creatinine appears to have a quenching effect on the intensity of triazolam. This was a common occurrence in many analyses. This was more evident in some drugs than for others, suggesting that creatinine is more easily ionized than some drugs, resulting in decreased drug intensity. For instance, **Figure 3-5** shows spectrum of oxazepam at 100 μg/mL in deionized water (top) and deionized water with 10 μg/mL creatinine (bottom), respectively.

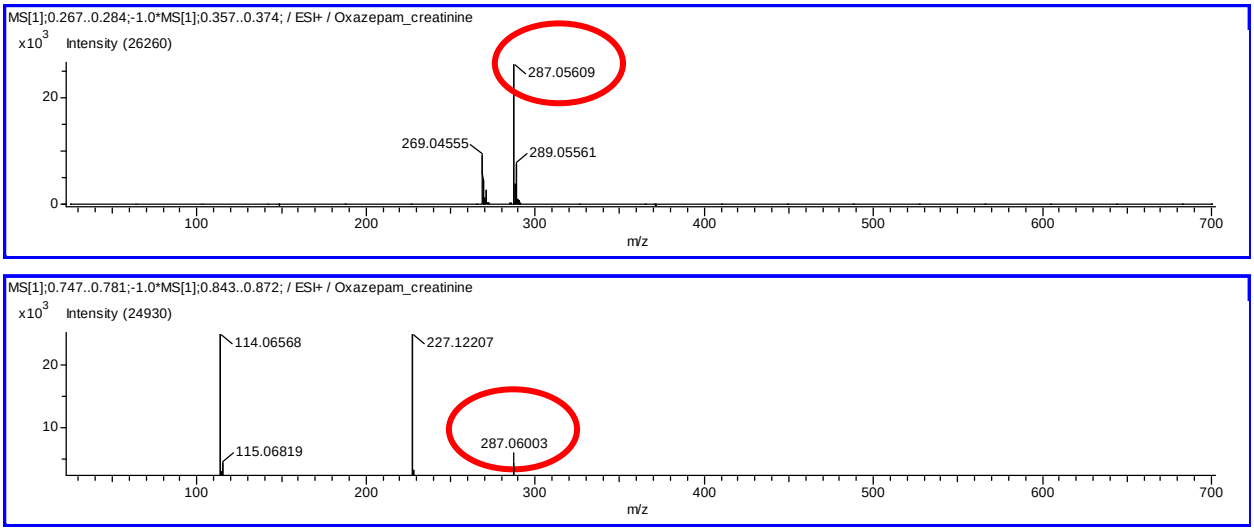

Figure 3-5. DART-TOF Spectrum of oxazepam at 100 μg/mL in deionized water and deionized water with 10 μg/mL creatinine.

Figure 3-5 shows a $(M+H)^+$ peak at high intensity in the top spectrum in contrast to that in the lower spectrum, in which oxazepam appears to be quenched by creatinine. When the creatinine concentration was increased to 100 μg/mL, the oxazepam peak approached nondetection. It appears that creatinine, which is present at high concentrations in urine, interfered with the ionization of most drugs.

Another occurrence that appears periodically is the variability of mass accuracy of drugs within the same peak. An example is provided in **Figure 3-6** with a spectra of a postmortem urine sample containing methadone and EDDP (methadone metabolite).

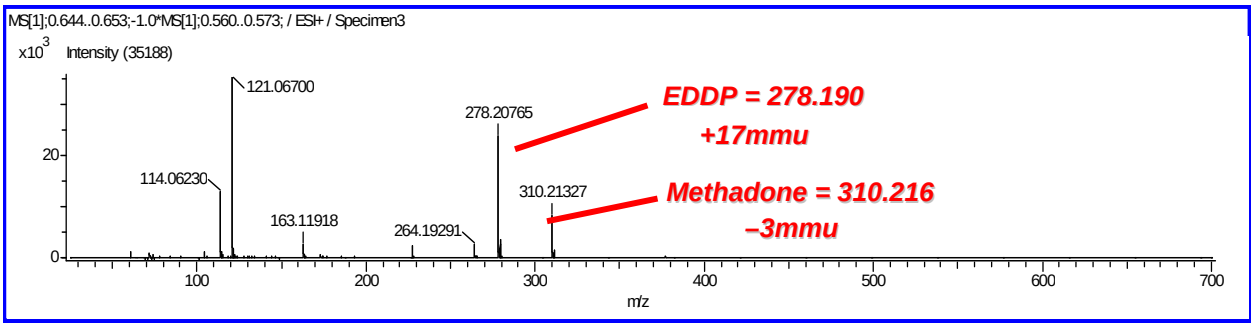

Figure 3-6. Spectra of variability of mass accuracy of methadone and EDDP within same run.

The observed $(M+H)^+$ value for EDDP was 17 mmu higher than the theoretical value, while methadone was only 3 mmu lower than the theoretical value of methadone. Both values were subjected to the same mass calibration. This phenomenon may be explained by the presence of an interference with a mass very close to EDDP, skewing its mass. This was also observed in standard materials and may be the result of variability in sample introduction. Such an occurrence would be problematic in samples in which the drug has not been identified and the presence of compounds is solely based on the mass. Thus, if the mass does not agree with the theoretical mass, it would not be identified and additional confirmation testing would not be pursued.

The DART-TOF system allowed for the rapid analysis of standard drug materials as well as urine specimens. Yet, analysis of authentic postmortem samples introduced several issues. The DART-TOF system achieved sensitivities for some drugs in urine that are relevant to postmortem toxicology overdose cases; however, appropriate sensitivity for other drugs was not attained. Interferences, such as creatinine, also resulted in lower sensitivity by quenching the ionization of compounds of interest. Mechanisms to separate drugs of interest from creatinine would be necessary to resolve this interference. Unfortunately, separation of this sort would also limit some of the advantage of the instrument because the sample preparation would then be similar to what is needed with other chromatographic methods. Lastly, isomers or compounds that are close in mass may cause interference problems, resulting in identification problems. While some of these compounds can be identified by fragmentation in isolation, when present in a mixture of an unknown sample, fragmentation is not as viable due to the likely presence of background peaks. Again, this increases the demand for sample preparation and decreases the advantage of this system over a traditional chromatographic analysis.

3.3 Phase II Analysis of Drugs in Blood and Tissue

Drugs in blood and tissue were examined both in spiked blood and in archived postmortem samples. Of the 50 drugs (Table 2-4) evaluated by DART-TOF, the following drugs in **Table 3-4** were successfully identified by DART-TOF in blood or tissue with or without sample extraction.

Table 3-4. Summary of Matrices Analyzed and the Drugs Successfully Previously Reported in Archived Postmortem Cases Used

Drug	Matrix	Amount (μg/mL)
Acetaminophen	Blood	12.5
Alprazolam	Blood	1.7
Amitriptyline	Liver	23
Caffeine	Blood	Positive
Carbamazepine	Blood	0.63
Carisoprodol	Blood	26
Cocaine	Blood	0.52
Dextromethorphan	Blood	1
Diazepam	Blood	0.26
Gabapentin	Blood	35
Mirtazapine	Blood	1
Meprobamate	Blood	3.5
Methadone	Blood/Liver	2.8/6.8
Methamphetamine	Blood	0.83
Nicotine	Blood	Positive
Nordiazepam	Blood	0.13
Oxcarbazepine	Blood	4.6
Propoxyphene	Blood	0.73
Quetiapine	Blood	8.6

Some example spectra are included in the following discussion. **Appendix C, Drugs in Blood,** provides a more extensive collection of example spectra from each class of drug from blood. This includes analyses after precipitation and after precipitation and concentration. **Figure 3-7** shows the spectrum of human blank blood analyzed by the DART-TOF.

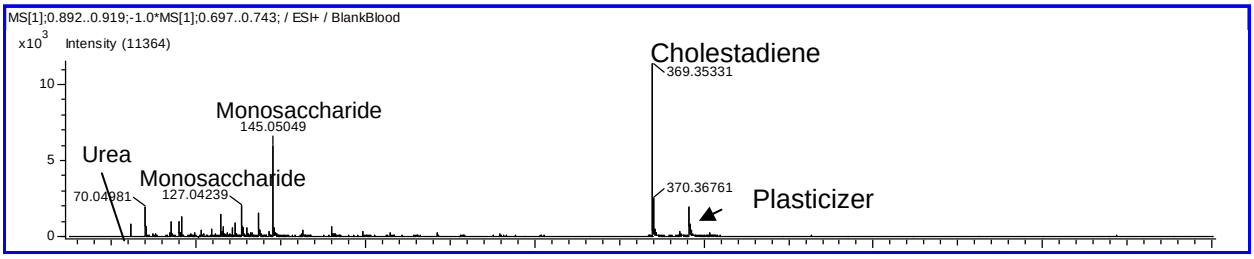

Figure 3-7. Spectra of human blank blood.

The common components present in blood, as seen in the spectrum, are urea, monosaccharides, and cholestadiene.

In most instances, postmortem blood and tissue analysis to detect drugs and toxins were improved by sample extraction techniques. As an example, methadone in blood (M+H ion 310.216) is easily detected in the unextracted sample; however, the extracted samples yield greater sensitivity, especially when they are concentrated (**Figure 3-8**).

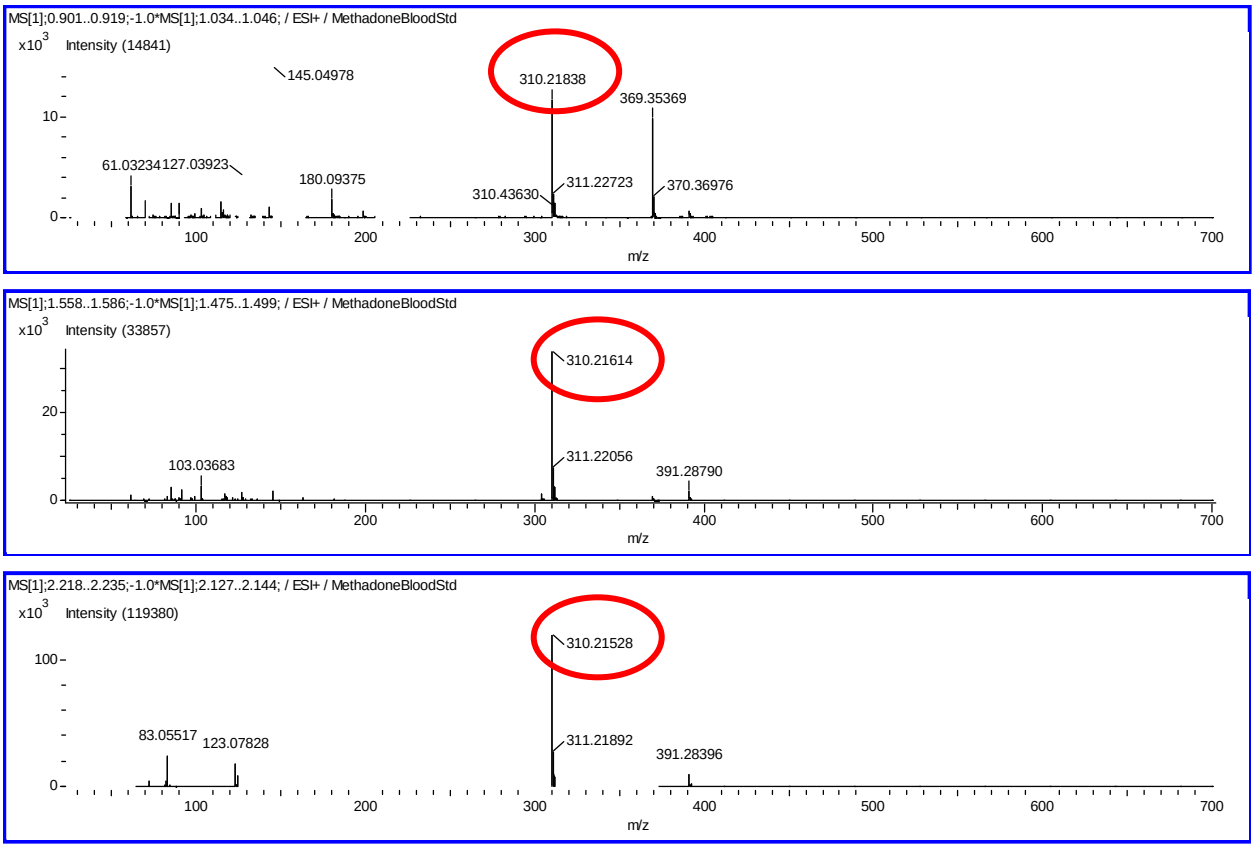

Figure 3-8. Spectra of methadone-spiked blood at 100 μg/mL unextracted, extracted in ACN, and extracted and concentrated.

Methadone was also detectable at 1 μg/mL when concentrated (**Figure 3-9**). Methadone was a compound more easily detected than many others (see Appendix C).

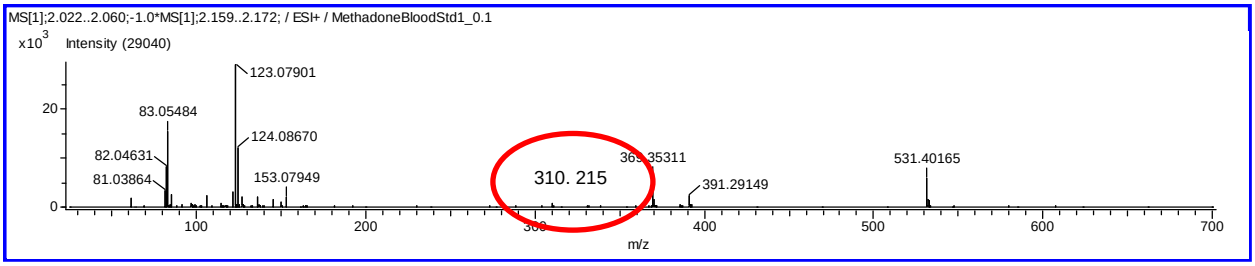

Figure 3-9. Spectra of methadone-spiked blood at 1 μg/mL.

Cocaine was also more easily detected, and a similar result was seen from the analysis of 1 μg/mL cocaine-spiked blood (**Figure 3-10**).

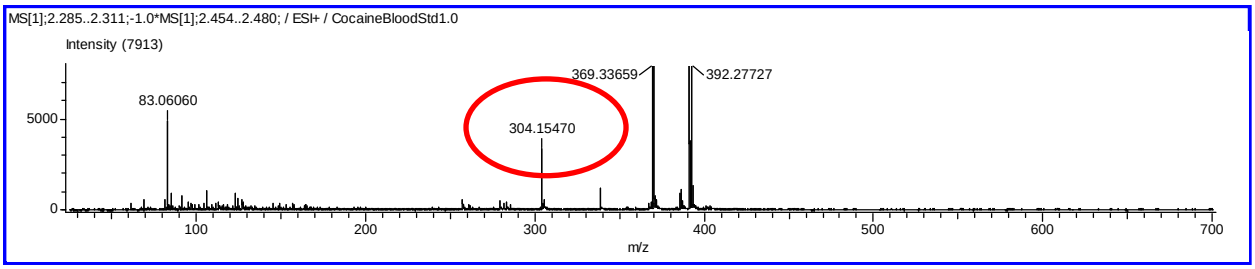

Figure 3-10. Spectra of cocaine-spiked blood at 1 µg/mL.

Common to most analyses, extraction and concentration was necessary to be able to detect target compounds. Analysis of many drugs in authentic postmortem blood proved undetectable at concentrations much higher than those detected in the spiked blood. **Figure 3-11** shows a postmortem blood specimen whose toxicology report showed 2.8 µg/mL of methadone present; however, without extraction methadone was not detected. This methadone concentration in postmortem blood was high and readily detected by traditional postmortem analysis. After extraction and concentration, methadone was detectable (**Figure 3-12**), similar to the detection of 1.0 µg/mL methadone from spiked blood above (**Figure 3-9**).

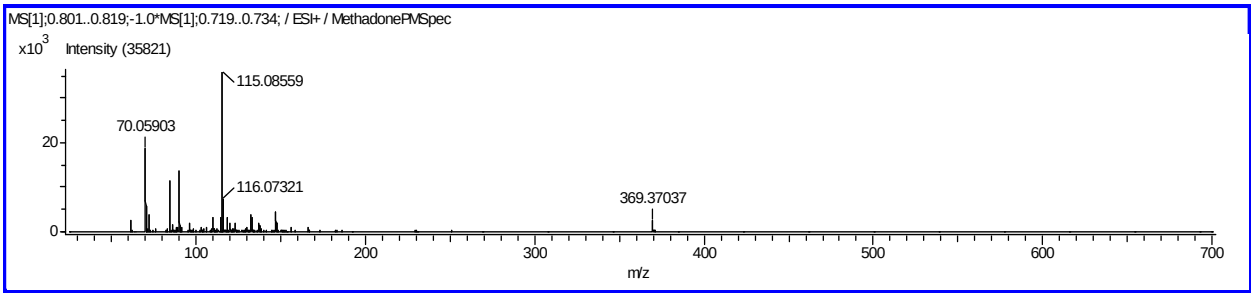

Figure 3-11. Postmortem blood specimen with methadone reported at 2.8 µg/mL undetected without extraction.

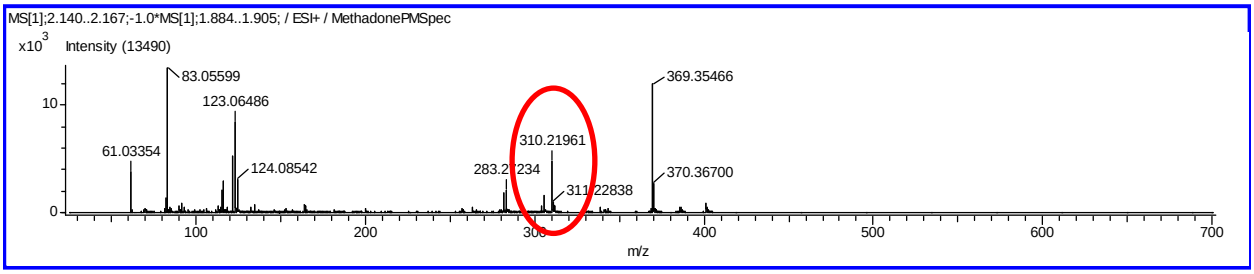

Figure 3-12. Methadone detected in the same postmortem sample after extraction and concentration.

The sensitivity was increased with the additional step of concentration. Not unexpectedly, the same results were found in tissue analysis. **Figure 3-13** shows the spectrum of an unextracted and extracted postmortem liver specimen determined to have 6.8 µg/mL of methadone by original analysis.

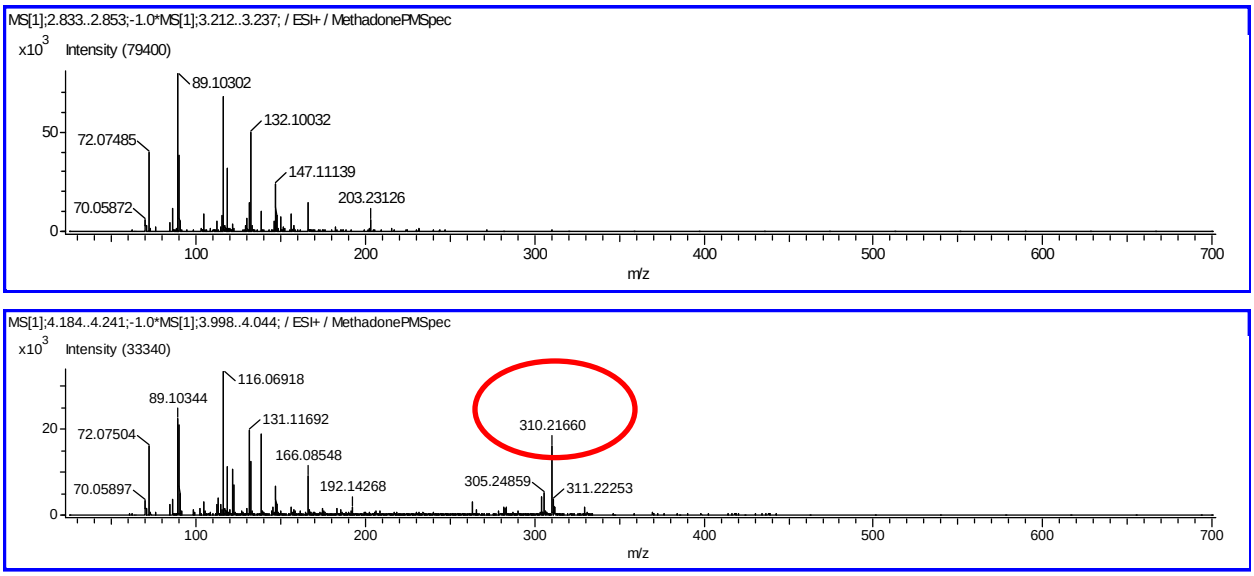

Figure 3-13. Spectra of postmortem liver specimen (reported at 6.8 µg/mL) unextracted and extracted and concentrated.

Minimal drug extraction needs to be performed in order to achieve any sensitivity for drugs of interest. The preparation removed interferences and concentrated the sample. Drugs detected in spiked blood had better sensitivity than in postmortem specimens at the same concentration. This may be due to more extensive protein binding in actual samples or the potential presence of more interference due to the degradation of the samples over time. This instrument is not ideal for detection of drugs in postmortem samples at therapeutic levels; however, the specimens' age may have contributed to compound degradation.

An ancillary investigation of two postmortem cases analyzed by both DART-TOF and other MS technologies was performed. In these cases, we were particularly concerned that previously confirmed drugs were not detected due to the age of the samples. **Figures 3-14** and **3-15** show DART-TOF analysis of the cocaine-containing case before and after SPE. **Figures 3-16** and **3-17** show DART-TOF analyses of the methadone-containing case before and after SPE. These samples were easily detected, and quantitative results were consistent with prior confirmation on more traditional LC/MS and MS/MS platforms. It was not evident that SPE preparation improved the DART-TOF sensitivity. From these data, it was evident that previously reported compounds were readily detected by these LC/MS and LC/MS/MS platforms. **Table 3-5** summarizes the detection and/or quantitation determined on each of the platforms used. Good agreement between our current quantitations with LC/TOF and LC/MS/MS with TOF as the third sector (LC/Q-TOF) in these cases and the previous quantitative results was obtained. There was evidence that the compounds previously reported in the samples were present in the analysis when the data were collected by Q-TOF, Ion Trap, and TOF. So the samples had not degraded in storage, and detection issues on DART-TOF were a result of sensitivity of the DART-TOF.

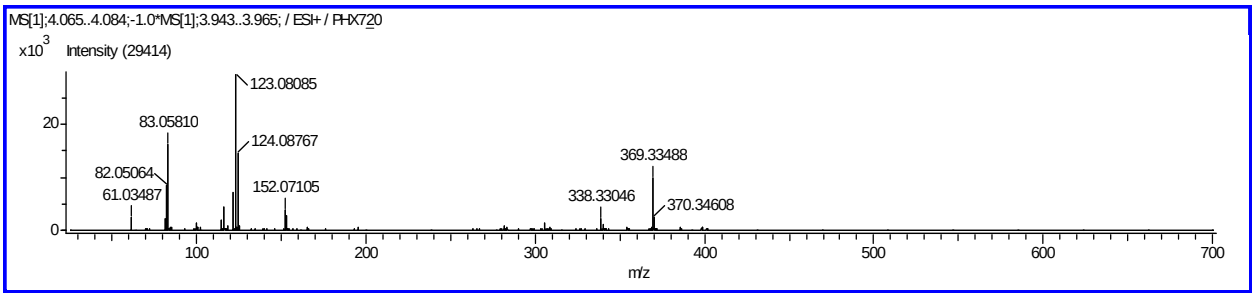

Figure 3-14. DART-TOF of the cocaine-containing case with acetonitrile extraction and concentration. No drugs were detected.

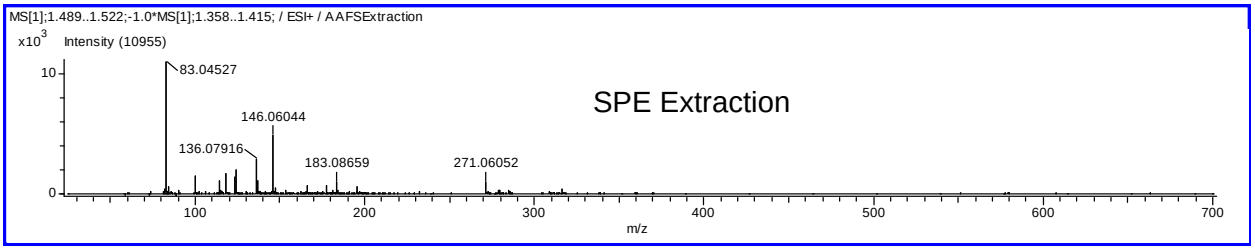

Figure 3-15. DART-TOF results of the same cocaine-containing case after solid phase extraction with no improvement of sensitivity for the compounds present.

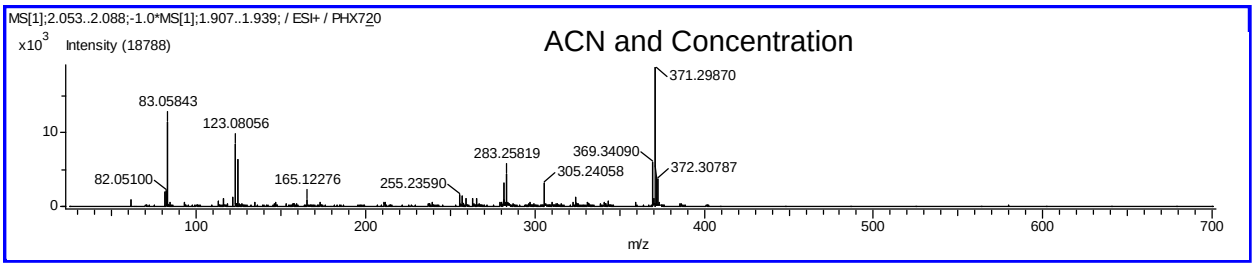

Figure 3-16. DART-TOF results of methadone-containing case with acetonitrile extraction and concentration. No drugs were detected.

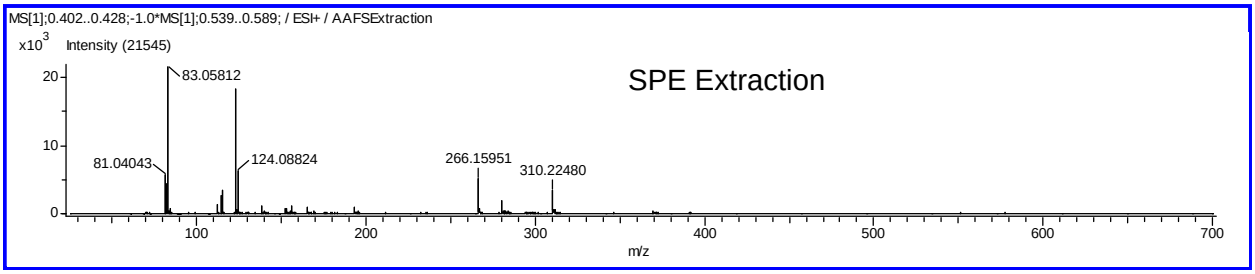

Figure 3-17. DART-TOF results of the same postmortem case previously confirmed for the presence of methadone. This spectra is from the direct introduction of the sample with solid phase extraction. No drugs were evident.

Table 3-5. Summary of the Quantitative and Detection Results for the Two Cases Analyzed on Multiple MS Platforms. Quantitations Were Consistent with Original Results from Traditional Platforms; However, the Compounds Were Not Detected by DART-TOF

	Case 1			Case 2
Platform	**COC**	**BE**	**CE**	**Methadone**
Original Quant	30 ng/ml	1,760 ng/ml	ND	1,100 ng/ml
LC/MS	8.8	1253	2.7	1156
LC/Trap*	Detected	Detected	Detected	Detected
LC/TOF	12.5	1632	6.4	754**
LC/QTOF	26.1	1539	10.4	898
LC/QQQ	1.1	1448	0.1	1134
DART-TOF	ND	ND	ND	

* No quantitation attempted on the trap

** Source was saturated, resulting in poor quantitation
ND = None detected

3.4 Discussion

Five LC/MS and LC/MS/MS technologies were able to detect the presence of previously confirmed compounds in the cases examined. While the DART-TOF's advantage was a simple and time-saving sample analysis, it did not have the sensitivity to detect these compounds in the cases examined. Comparable quantitative results were obtained for all LC-based platforms, except the LC-Trap system. While the intention of the DART-TOF analysis was not to evaluate quantitation, the quantitative results from the LC-based MS platforms provided information that the samples were reasonably stable and representative of their original results. With DART-TOF, some successful identification of compounds previously reported in samples was obtained; however, this was unpredictable and incomplete. The comparison illustrated that for biological samples, the relative insensitivity of the DART-TOF precludes its being currently applicable to biological samples, except for those where very high concentrations might be expected. These findings indicate that DART-TOF's rapid and simple analysis merits work toward improving the sensitivity.

3.5 Stage IV: Additional Study Comparing DEA Cocaine Signature Analysis to DART-TOF Analysis

Table 3-6 contains the theoretical M+H values of the target analytes that were detected in the cocaine exhibits by DEA Cocaine Signature Analysis. This analysis comprises multiple assays to evaluate the presence and concentration of various cocaine analytes, adulterants, and solvents among other components in illicit cocaine.

All values are reported to the millimass unit (mmu), with the exception of petroleum, which has a very low mass, and thus a larger expected mass error. The results of the DART-TOF analysis of the 25 cocaine samples in comparison to the multi-technique signature analyses are shown in **Table 3-7**.

Table 3-6. Theoretical Monoisotopic Mass + H of Analytes

Analyte	Theoretical Monoisotopic Mass + H
Anydroecgonine methyl ester	182.117
Benzoylecgonine	290.139
Caffeine	58.958
Cinnamoylcocaine	330.169
Cocaethylene	318.169
Dimethylterephthlate	195.064
Ethyl acetate	89.052
(Iso/n-)propyl acetate	103.068
Lactose	343.116
Mannitol	303.079
Methyl ethyl ketone	73.064
Methyl isobutyl ketone	101.096
Norcocaine	290.138
Petroleum ether	87-90
Sodium chloride	58.985
3,4,5-trimethoxycocaine	393.178
Tropacocaine	246.141
Truxillines	659 (numerous isomers)

Table 3-7. The Number of Samples, Out of the Total 25 Analyzed, that Tested Positive for the Various Analytes, Using the DART-TOF System and Cocaine Signature Analysis

Analytes	Cocaine Signature Analyses	DART-TOF
Anhydroecgonine methyl ester	ND	23
Benzoylecgonine	21	25
Cocaethylene	7 (trace)	ND
Cinnamoylcocaine	25	23
Norcocaine	21	25
3,4,5-Trimethoxycocaine	25	ND
Tropacocaine	25	5
Truxillines	25	7

ND = none detected

AEME and cinnamoylcocaine were easily detected by DART-TOF in 23 out of the 25 samples as shown in the DART-TOF spectra depicted in **Figures 3-18** and **3-19.**

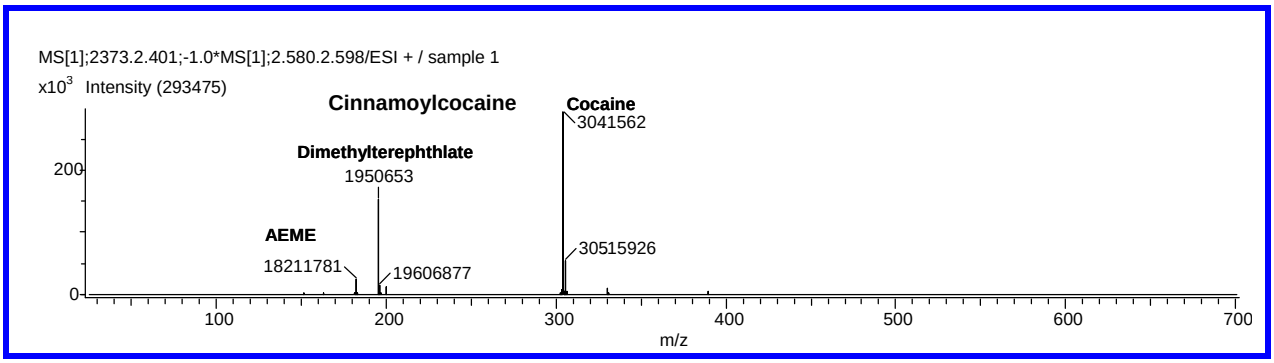

Figure 3-18. DART-TOF spectra of an illicit cocaine sample showing the presence of cocaine, anhydroecgonine methyl ester (AEME), dimethylterephthlate, and cinnamoylcocaine.

In all samples, there was an ion present at 290.139 *m/z*, which is the M+H value of $C_{16}H_{19}NO_4$. This is the molecular formula of the isomeric pair benzoylecgonine (BE) and NCOC, which have indistinguishable masses. Figure 3-19 shows the presence of the ion at 290.169 in an analyzed sample.

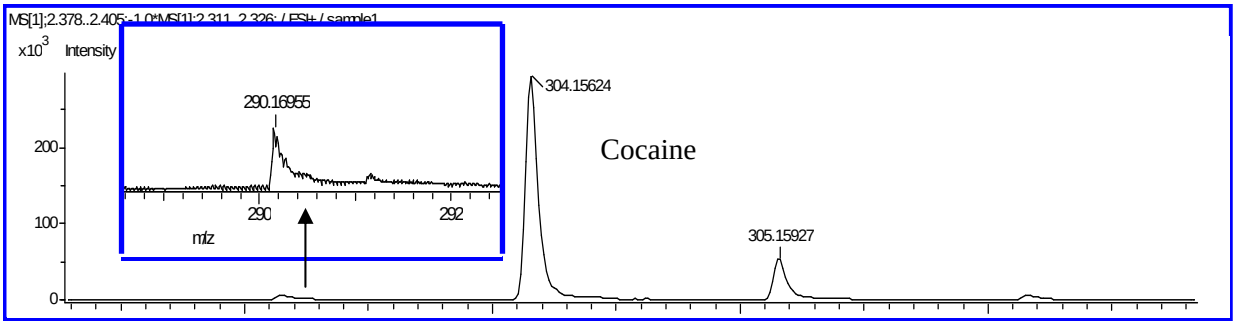

Figure 3-19. DART-TOF spectra of an illicit cocaine sample showing the presence of possible norcocaine and BE isomer (290.169).

Although the difference of 30 mmu is not optimal, it may be due to an interference present at a similar mass, resulting in a skewed *m/z* value. This was a problem encountered during DART-TOF analysis of biological samples and drug materials. For example, known analytes may be analyzed sequentially and subjected to the same calibration, but one peak will generate an M+H value of 1 or 2 mmu from its theoretical value and the other will have a difference of more than 10 mmu. Cocaine has a theoretical M+H value of 304.154 and, as seen in the analysis of a sample in Figure 3-19, is only 0.002 mmu higher than expected; this is not the case with the BE/NCOC isomer. While we were able to fragment BE to distinguish it from NCOC previously, this was done with methanolic standards at a high concentration and was unsuccessful when analyzing the cocaine samples. Tropacocaine and truxillines were present in **Figure 3-20,** while 3,4,5-trimethoxycocaine and cocaethylene were undetected.

AFIP's analysis of the cocaine materials produced similar results for NCOC to DEA. However, AFIP's results for other compounds were not consistent, and difficulties in their analytical procedures led us to only use their NCOC results for comparison.

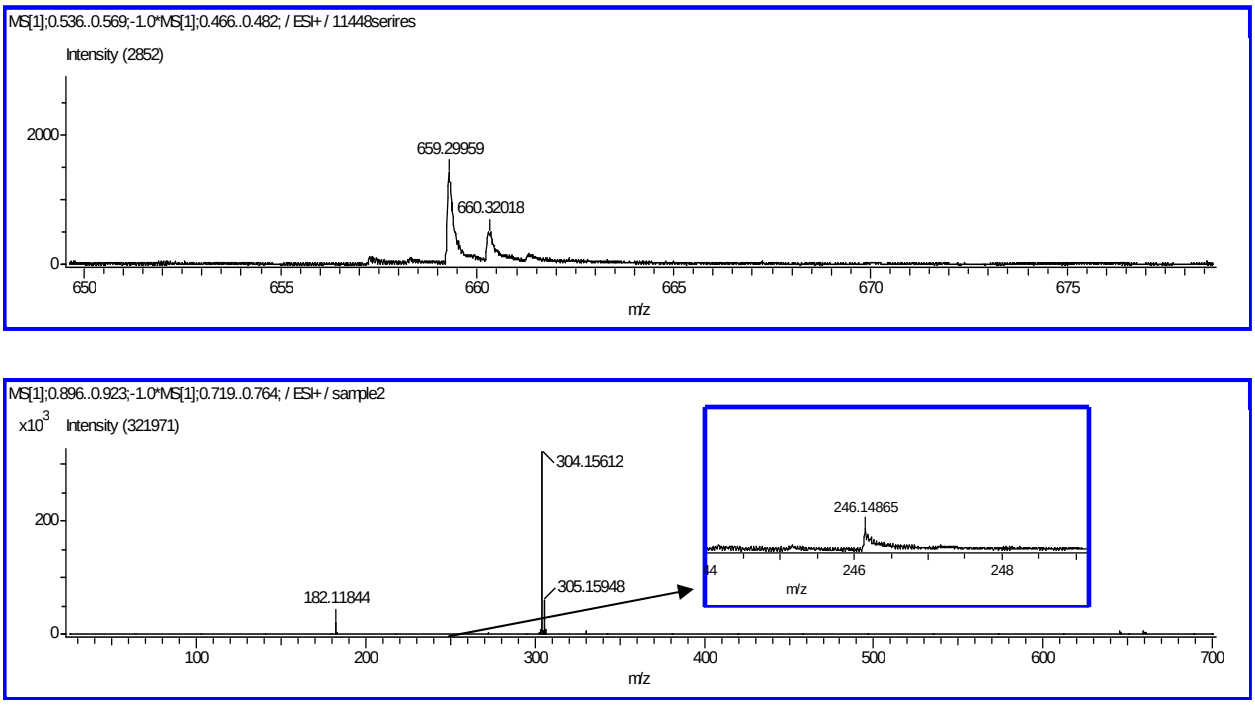

Figure 3-20. DART-TOF spectra of an illicit cocaine sample showing the presence of truxillines (659.29959) and tropacocaine (246.14865).

Of the solvents and adulterants/diluents detected by DEA, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), and dimethylterephalate were identified by the DART-TOF.

The DART-TOF allowed for the rapid introduction and analysis of 25 illicit cocaine samples without the need for sample preparation. Although this direct analysis resulted in rapid production of data, it also caused inconsistent results. Since the introductions of the powdered samples were done manually, without the use of an autosampler, the outcome was analyst-dependent. Many samples were analyzed multiple times in an effort to verify the presence or absence of the analytes. Although analytes such as AEME and cinnamoylcocaine were easily detected in most of the samples, others such as tropacocaine and 3,4,5-trimethoxycocaine were minimally detected, if at all.

The DART-TOF is a novel approach to forensic analysis; however, the analysis of crude illicit cocaine in this study proved ineffective for detecting the presence of many compounds that would be used to trace a cocaine sample to its geographic origin. In an effort to increase laboratory production, forensic laboratories may use the DART-TOF as a rapid screening test for preliminary sample-to-sample comparison work, which could then be confirmed by a more thorough investigation, such as signature analyses.

Potentially, a similar situation to the creatinine interference in urine may play a role in the analysis of cocaine in this circumstance. The presence of large quantities of cocaine may interfere with the ability of the lower concentration components to ionize, and thus limit their detection. This may explain some of the differences between the low concentration analytes reported by DEA and the nondetection of these compounds on DART-TOF.

3.6 Discussion of the Equipment and Software Performance

3.6.1 DART-TOF System

The DART-TOF system provided rapid analysis of samples used in this study. There were two issues; however, that affected the quality of results. The first has to do with the open sample gap on the ion source. Use of some solvents in the laboratory interfered with the analysis. Particularly, ethyl acetate could not be used to concentrate samples because the vapors lingered and would appear in the analysis to the point of obscuring other compounds. The second issue was, as discussed previously, the variability of mass accuracy of drugs within the same analysis peak. We have been unable to pinpoint why mass accuracies have been periodically unpredictable. Other DART-TOF users have experienced similar observations.

Overall, the system was easy to operate with minimal maintenance. In the course of the study (12 months), we only had to clean the O1 plate two times and did not notice any diminished performance of the instrument between cleanings. The microchannel plate detector remained responsive at the same voltage that we originally started with on the instrument. We have experienced no hardware failures to date.

3.6.2 Instrument Software

There were several issues concerning the software for the system, Mass Center. First, due to the number of windows (at least six) that are open during acquisition, a second computer screen was needed to reduce crowding and improve viewing. This made navigating the various windows overwhelming at first. In addition, windows minimize and layer over each other in unexpected ways, making navigation difficult to learn.

The software appears to have some instabilities resulting in error messages, such as “Stop acquisition because temperature NG.” However, all indications were that temperatures were appropriate on the instrument. The problem was intermittent, with no apparent resolution. In addition, the chromatogram screen would freeze periodically during acquisition. Although this would occur if the project data file folder contained too many files, it would also happen sporadically when using a new project folder. Again, the problem was intermittent, and the size of project folder at which problems arose was not consistent. Again, there is no apparent resolution.

Because the setup to start a sample acquisition requires many multiple steps, we resorted (as many users do) to collecting multiple samples within an individual run. This necessitates the analyst to keep written track of which samples are being introduced. This is unlike commonly used GC/MS or LC/MS data acquisition software, which stores each introduced sample as a separate data file. This creates problems for data handling and the forensic presentation of data. Overall, the software took more time to learn to navigate than other mass spectrometry software that most analysts had used.

3.6.3 LEAPShell Autosampler

The LEAPshell autosampler worked well with the DART system by providing consistent sample introduction that yielded optimal peak shape as opposed to manual introduction, which many times required repeat analysis. There was little maintenance involved, and the software was easy to operate.

4. CONCLUSIONS

Overall, the DART-TOF system has potential for use in the screening of biological samples but currently is not sensitive enough to be useful in most cases. While many compounds can be detected at high concentrations out of standard solutions, the analysis of these same compounds in biological matrices at concentrations that are relevant to the majority of cases is erratic. Interferences from other common components, such as urine creatinine, limit sensitivity by the apparent quenching of ionization. Likewise, the mass accuracy can be affected by the matrix and other undetermined factors, which, in an unknown sample, could result in undetected compounds. Isomers, too, are not always distinguishable in isolation by fragmentation, and in a biological matrix of an unknown sample, fragmentation is likely not viable. The potential for interferences from the matrix and the need to concentrate samples results in the need for sample preparation (extraction and concentration) equivalent to more traditional analysis methods.

The instrument has problematic software that demands a high degree of operator input. While this can be accommodated, the software is also unstable and nonstandard in its operation. The manufacturer is aware of the problems and has taken some steps to correct problems in the software. Also, the forensic presentation of the data obtained may be challenging because of the need for a high degree of manual input and the lack of auditing; the demand for explanation of the data likely would be high.

Despite the current drawbacks of the instrument, the potential for extremely rapid analysis and broad detection ability merits further work. The instrument may have potential as a postmortem screening instrument, but further research is needed to improve sensitivity. For drug chemistry screening, it has current potential because sensitivity is not as great an issue for this type of application. It should be kept in mind that this is essentially a first-generation instrument. It is a significantly different concept in instrumentation than current screening methods and is the first to offer minimal-to-no sample preparation combined with broad screening capabilities. Additional efficiencies may be obtained in more traditional screening technologies, such as immunoassays or LC/MS, GC/MS, or GC/NPD, but chromatographic separation and immunoassay have limitations in sensitivities to fixed, cross-reacting classes or have limitations to how rapidly the method may perform. If the sensitivity of the DART-TOF system can be improved, it could have a significant impact on laboratory throughput and data quality. The additional issue of software problems can be resolved by the manufacturer to further improve the DART-TOF functionality for forensic applications.

4.1 Implications for Policy and Practice

Toxicology laboratories are often backlogged, creating budgetary and policy problems. In fact, continuing concern over this issue has inspired the Coverdell Grant program to improve the quality and timeliness of forensic laboratories. Backlogs delay and potentially compromise investigations and delay payment of death benefits to survivors. Backlogs can result in case information not being available when needed and may require decisions to limit toxicology work. Technologies that reduce screening time requirements, while increasing the range of analytes detected, will provide higher quality death investigations at reduced costs.

The DART-TOF system appears to have a potential application to forensic analyses; however, at the present time the issue of sensitivity limits its applicability. The instrument has current applications, such as drug chemistry, for which it likely could improve the workflow. As a rapid screening tool, it could impact backlogs in drug chemistries. Currently, it likely would not have much impact on toxicology backlogs because the sensitivity would require a sample workup similar to that required for more traditional chromatographic analysis.

The software issues need to be addressed before the instrument will be widely accepted. A much more simple and traceable relationship between data collection and derived data is necessary to prevent

challenges to the data that could require an extensive explanation for legal purposes. The software needs to be modified to promote stability and standardization in its performance as well as to be less labor intensive.

5. DISSEMINATION OF RESEARCH FINDINGS

The research findings of this project have been presented at the following annual meetings:

(1) Miller, J.D.R., E.J. Minden, N.D. Bynum, P.R. Stout, J.F. Casale, I. Kim, J. Runkle, M. Past, and B.D. Paul. 2008. *Signature Analysis of 25 Illicit Cocaine Samples and a Comparison to Analysis by DART-TOF*. American Academy of Forensic Sciences (AAFS). Washington DC, February 18–22.

(2) Stout, P.R., and J.D. Ropero-Miller, *Evaluation of Direct Analysis Real Time of Flight Mass Spectrometry (AccuTOF DART) for Postmortem Toxicology Screening.* 2007. NIJ Grantees' Meeting: RTI International Forensic Research Programs. AAFS. San Antonio, TX, February 19–24.

(3) Stout, P.R., and J.D. Ropero-Miller. 2008. *Evaluation of Direct Analysis Real Time of Flight Mass Spectrometry (AccuTOF DART) for Postmortem Toxicology Screening.* NIJ Grantees' Meeting: RTI International Forensic Research Programs. AAFS. Washington DC, February 19–24.

(4) Stout, P.R., N.D. Bynum, E.J. Minden, Jr., and J.D.R. Miller. 2007. *Evaluation of Urine Samples Utilizing Direct Analysis Real Time of Flight Mass Spectrometry (AccuTOF DART) for Postmortem Toxicology Screening.* Society of Forensic Toxicologists (SOFT). Raleigh-Durham, NC, October 14–19.

(5) Minden, E.J., Jr., N.D. Bynum, J.D. Ropero-Miller, and P.R. Stout. 2007. *Establishment of a Drug Standard Reference Library for Postmortem Toxicology Using Direct Analysis in Real Time (DART) Time-of-Flight Mass Spectrometry (TOF-MS).* SOFT. Raleigh-Durham, NC, October 14–19.

The results of the study are also available as on-demand content as part of the Web-based continuing education program funded under NIJ cooperative agreement 2007-DN-BX-K208. A presentation of the DART-TOF project, as well as the comparison of MS platforms, is available at www.rti.org/forensiced under the recent AAFS presentations class. As of April 28, 2008, this material has had approximately 90 registered participants.

In addition, RTI has made the dissemination of these research findings via publications a priority of the project. The following publication is currently in print:

(1) Published Manuscript:
Ropero-Miller J.D., P.R. Stout, N.D. Bynum, and J.F. Casale. 2007. Comparison of the novel direct analysis in real time time-of-flight mass spectrometry (AccuTOF-DART) and signature analysis for the identification of constituents of refined illicit cocaine. *Drug Enforcement Administration Microgram Journal 5*(1–4):34–40.

6. IMPLICATIONS FOR FURTHER RESEARCH

The most significant need for further research on the DART-TOF system is to improve the instrument's sensitivity. While some sensitivity gains were demonstrated in this study by using some simple sample preparation techniques, ultimately the instrument needs to be more sensitive as a screening tool. There is potential to utilize the DART source on other MS platforms, and the addition of a MS/MS platform could improve sensitivity significantly. Additionally, some preliminary work from Edgewood Chemical Biological Center on modifications to the source may also provide needed improvements to sensitivity (personal communication). Further testing and development of such technologies is essential.

The initial comparison of cocaine analysis to DEA's cocaine signature program was promising and indicates that the DART-TOF has strong current potential for drug chemistry screening. Additional work to compare results from multiple facilities on drug chemistry cases is essential to address questions about the reproducibility of results. Additional work of this kind that results in publication is essential for the acceptance of the instrument in court and to its successfully being admitted through a "Daubert challenge."

Software development and development of spectra libraries and search software is also an important component of future work with the instrument. Understanding the workflow of practicing laboratory settings and understanding the demands placed on the data are essential to improving the utility of the software. The software needs to be capable of successfully operating the instrument, but also producing auditable data that can be clearly explained. Examining the data with the understanding of how it will be used would provide a significant improvement in reducing the likely challenges to the data.

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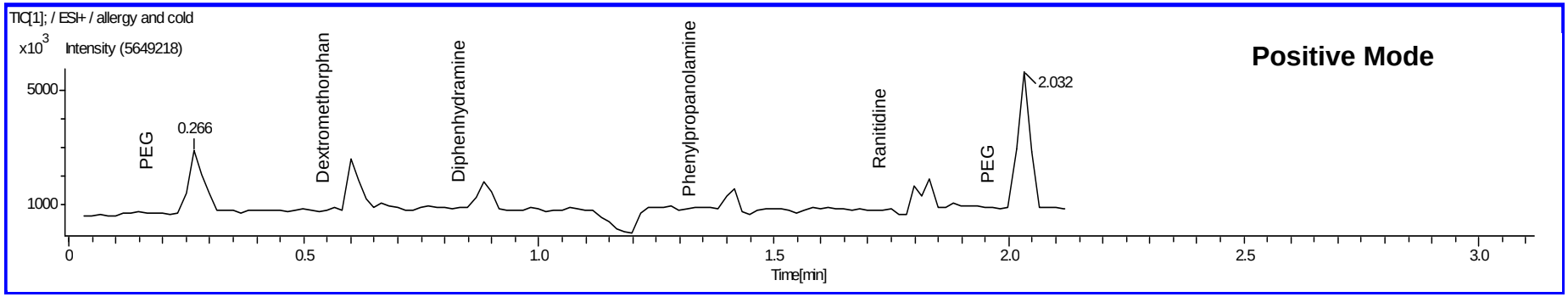
Appendix A

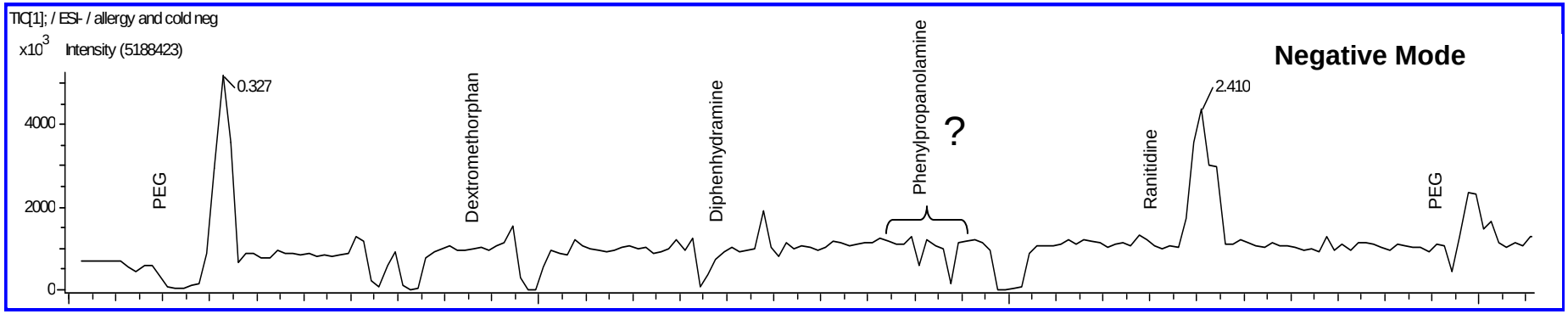
Methanolic Drug Standards

Appendix A

Methanolic Drug Standards

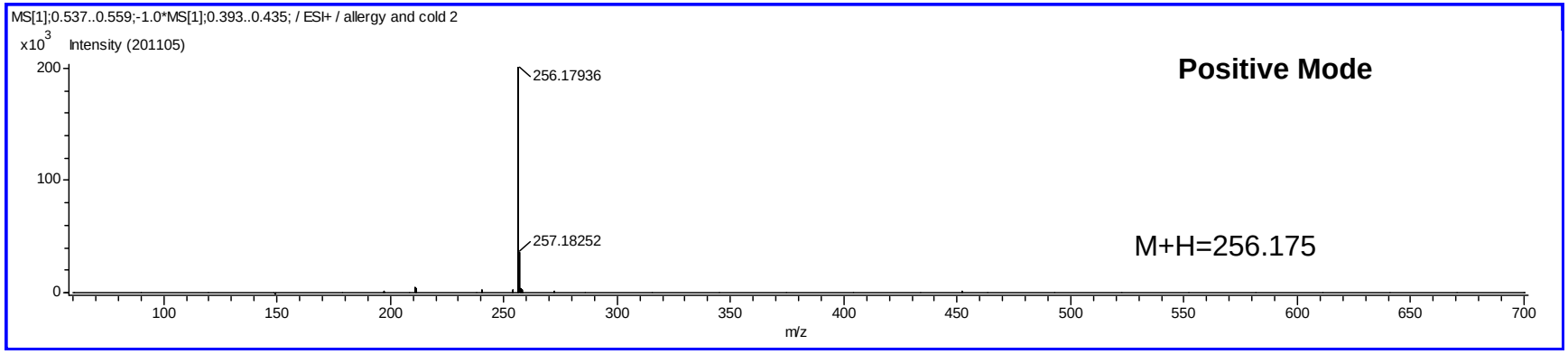
A-1. Allergy/Cold (4/25/07)

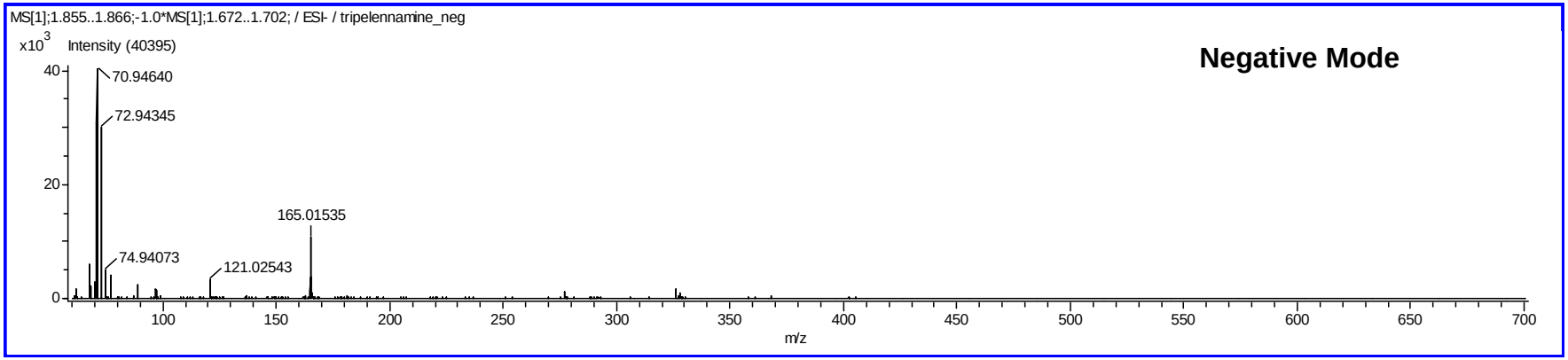




A-1. Allergy/Cold (continued)

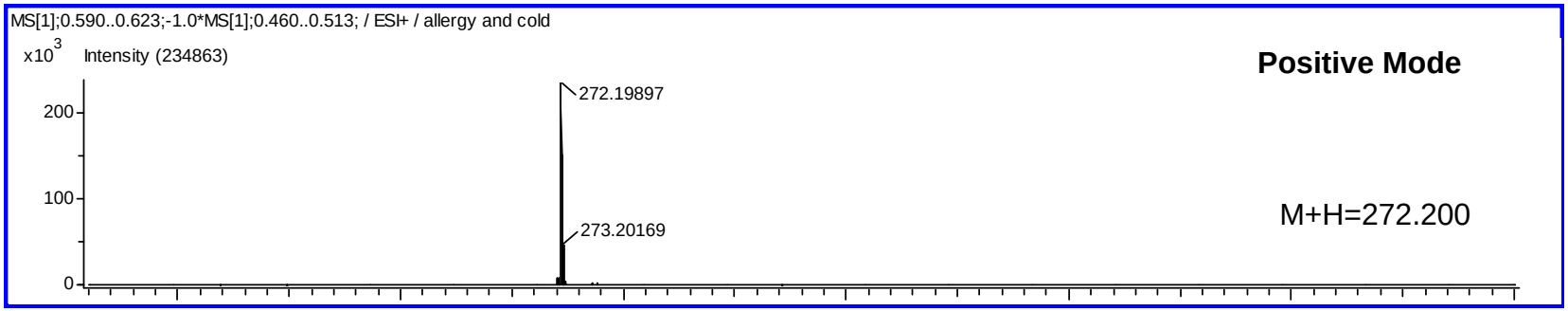
A-1.1. Tripelennamine

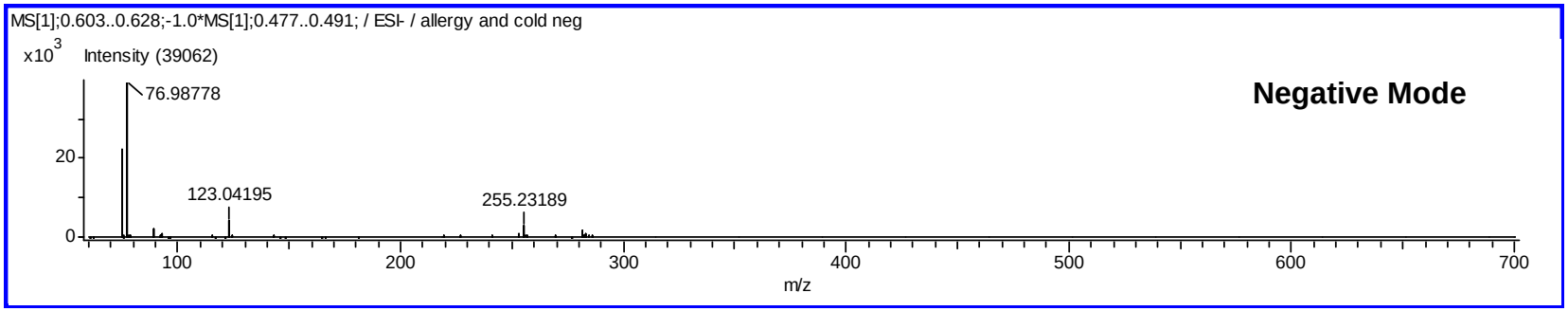




A-1. Allergy/Cold (continued)

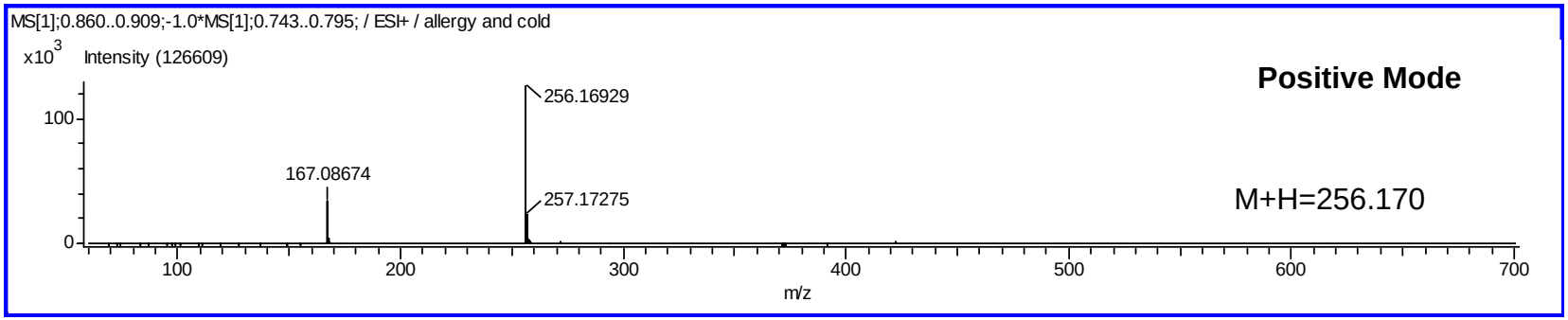
A-1.2. Dextromethorphan

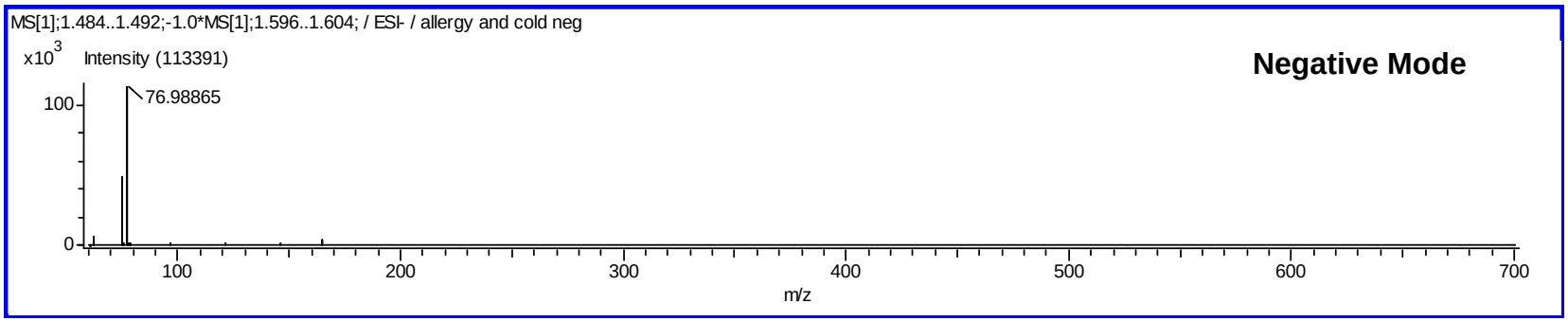




A-1. Allergy/Cold (continued)

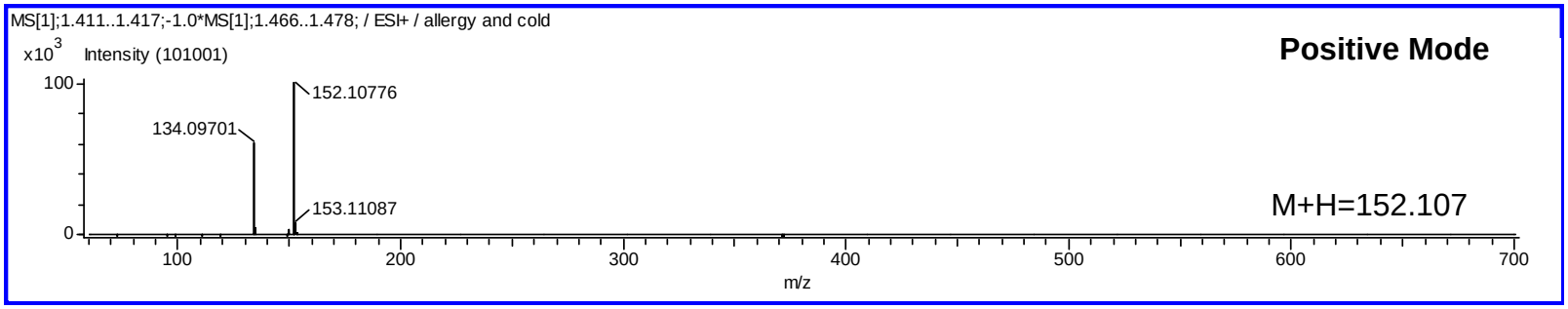
A-1.3. Diphenhydramine

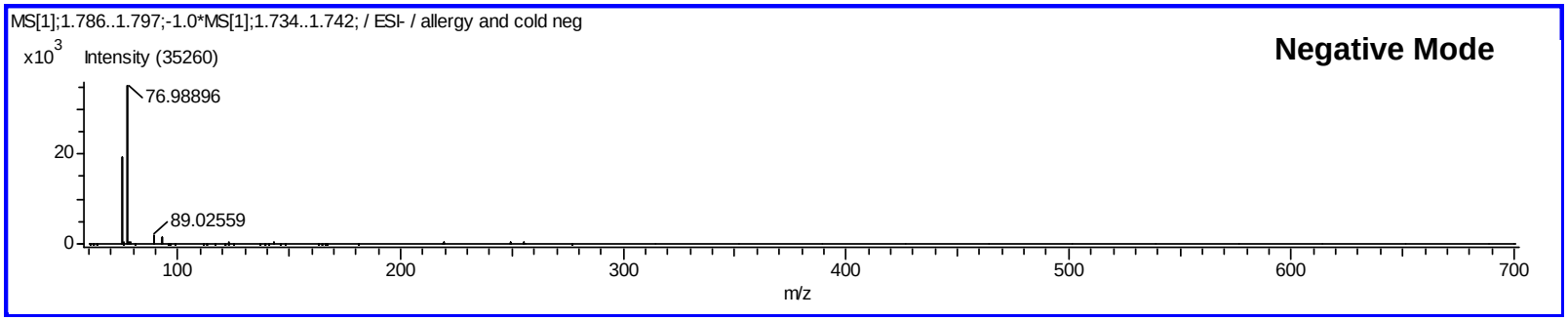




A-1. Allergy/Cold (continued)

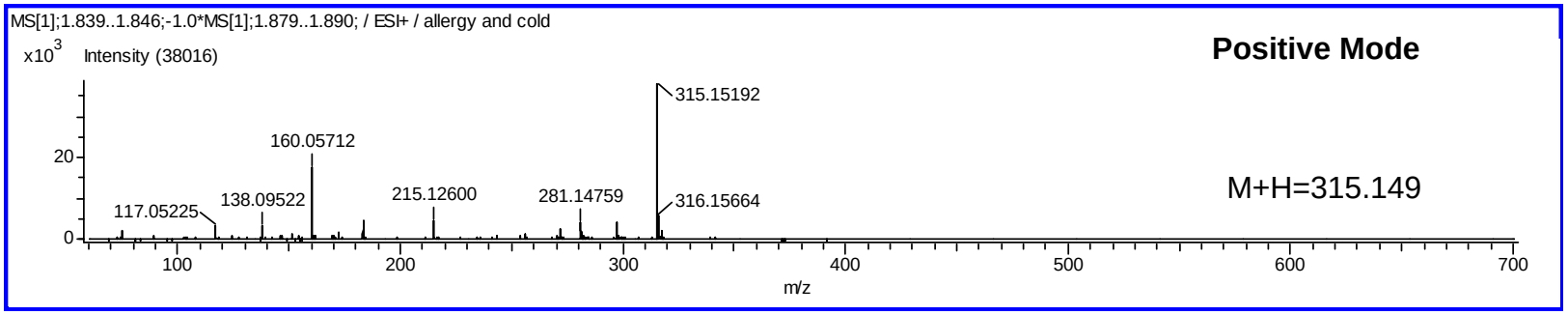
A-1.4. Phenylpropanolamine

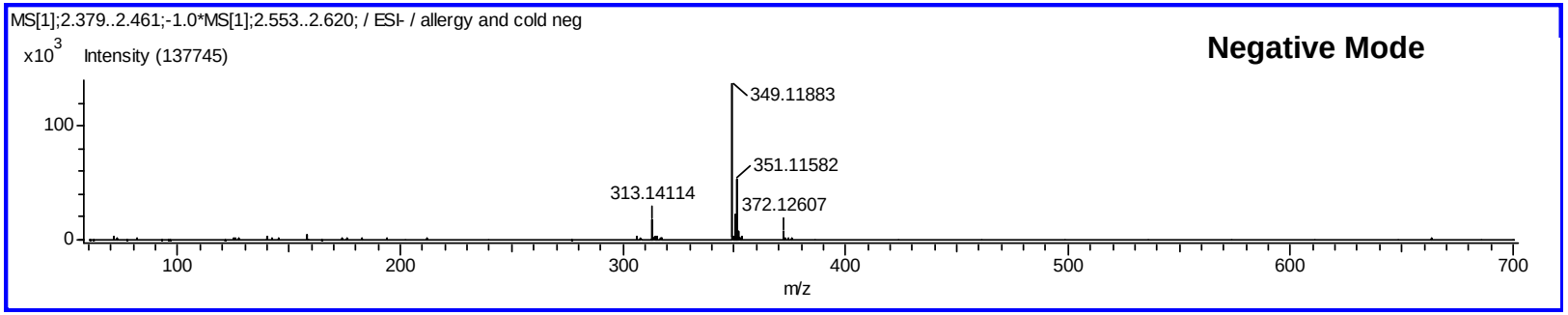




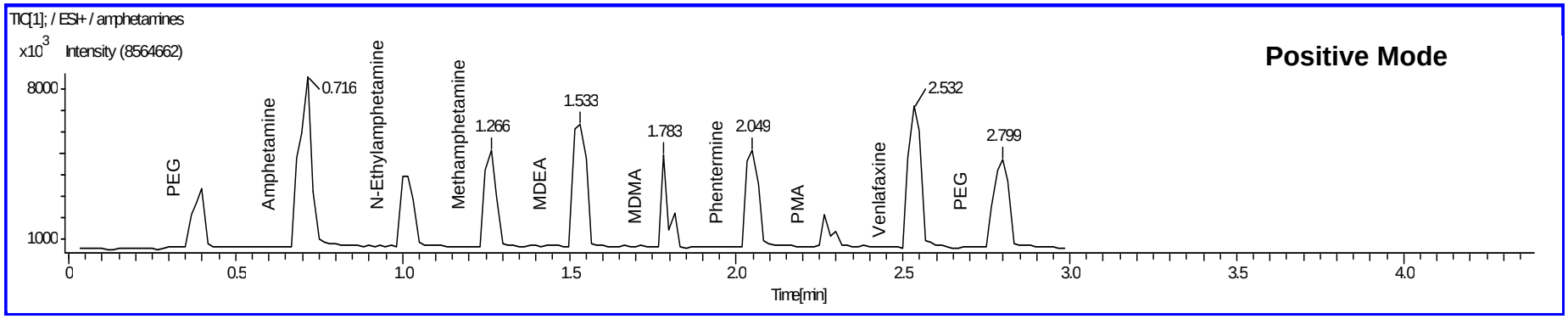
A-1. Allergy/Cold (continued)

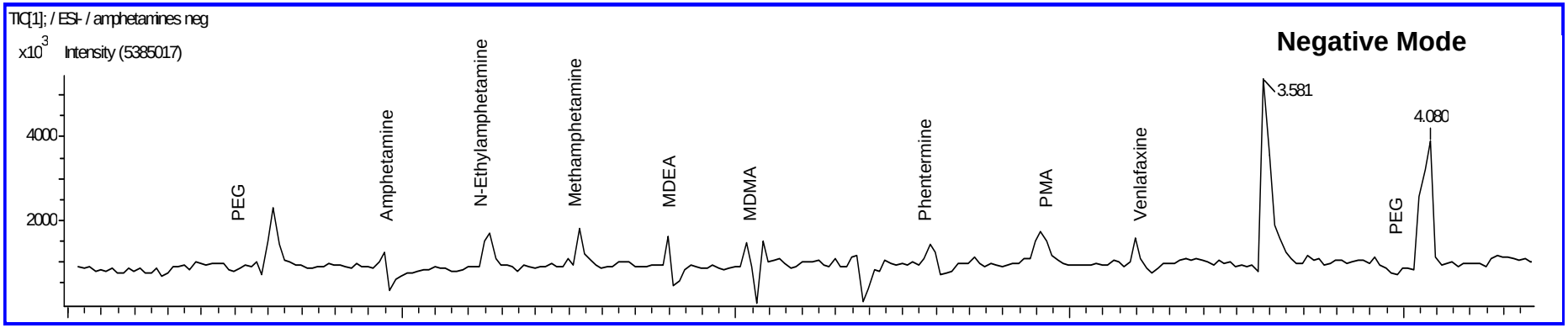
A-1.5. Ranitidine





A-2. Phenethylamines (4/26/07)



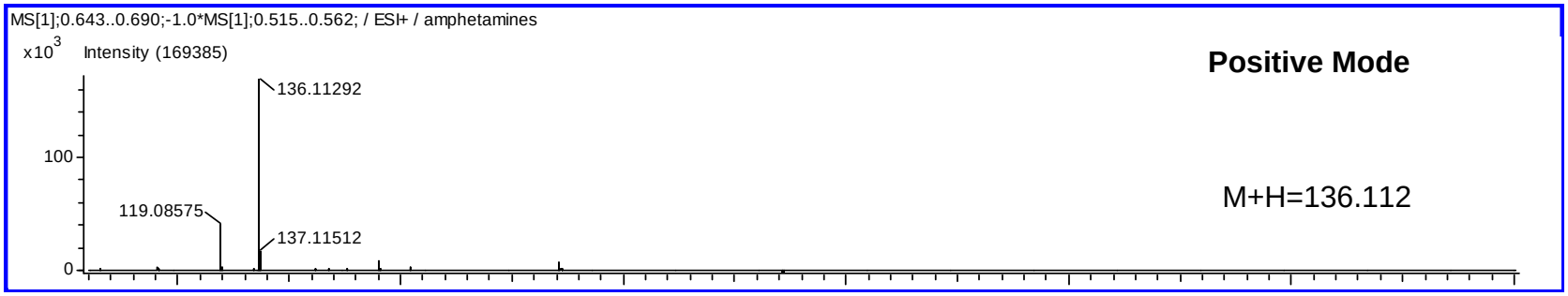


Drugs did not work in negative mode.

Peaks 76 and 165 present.

A-2. Phenethylamines (continued)

A-2.1. Amphetamine



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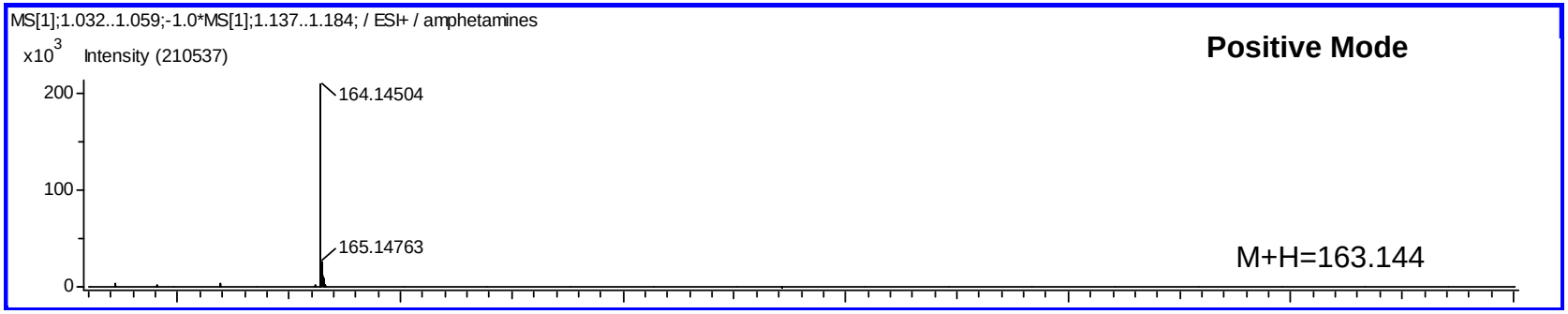
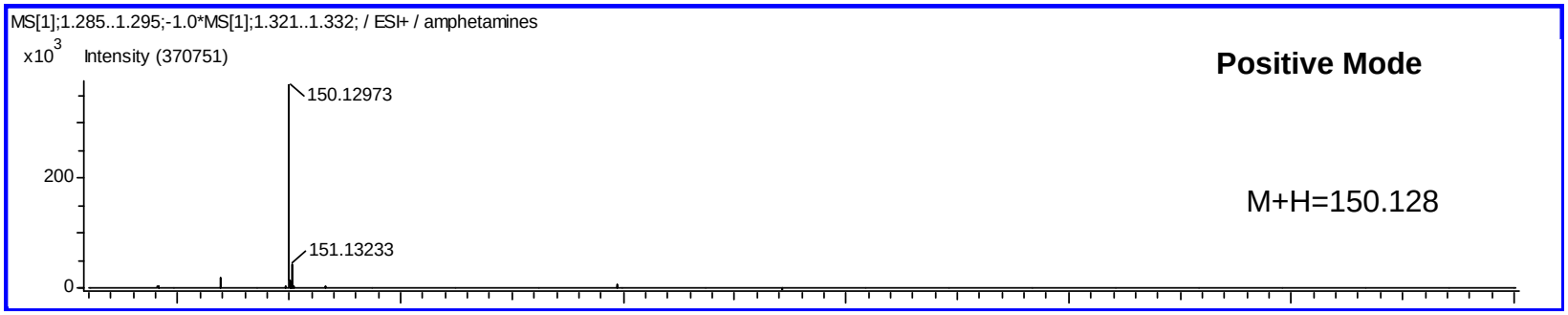
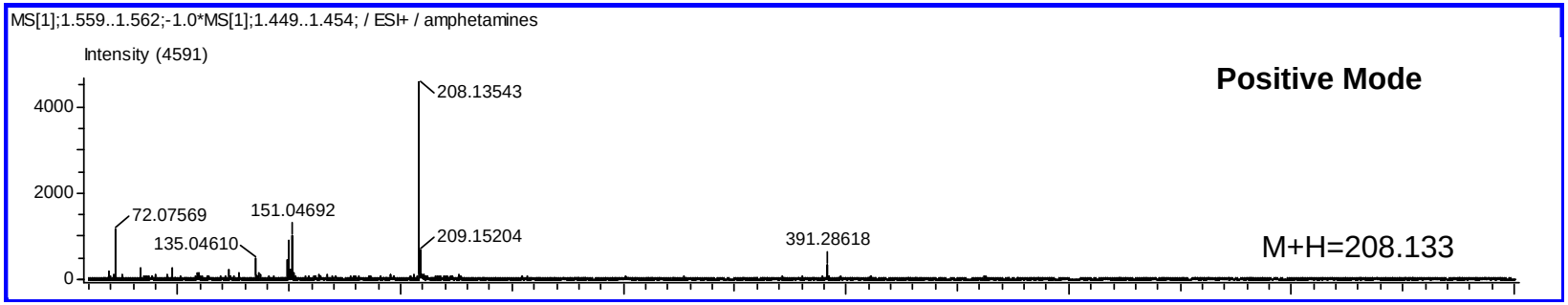


A-2. Phenethylamines (continued)

A-2.3. Methamphetamine

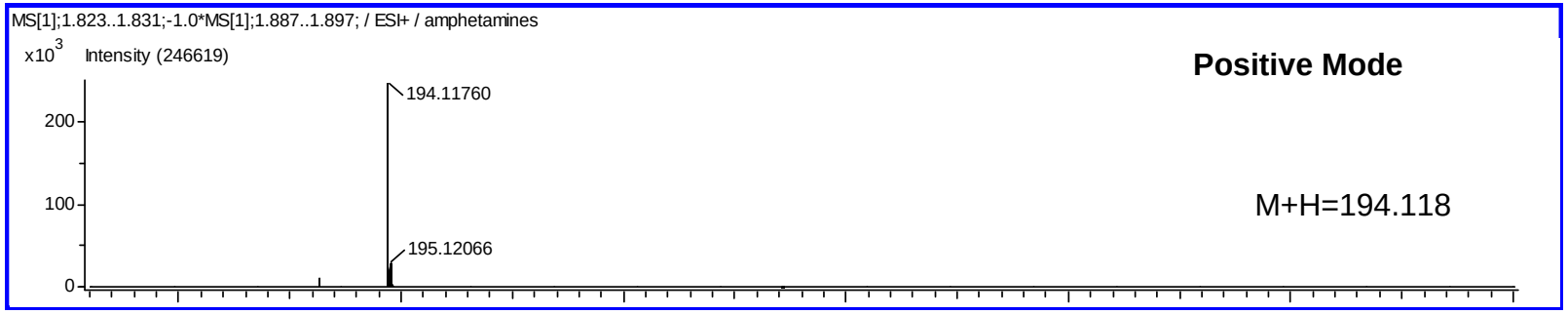


A-2.4. MDEA

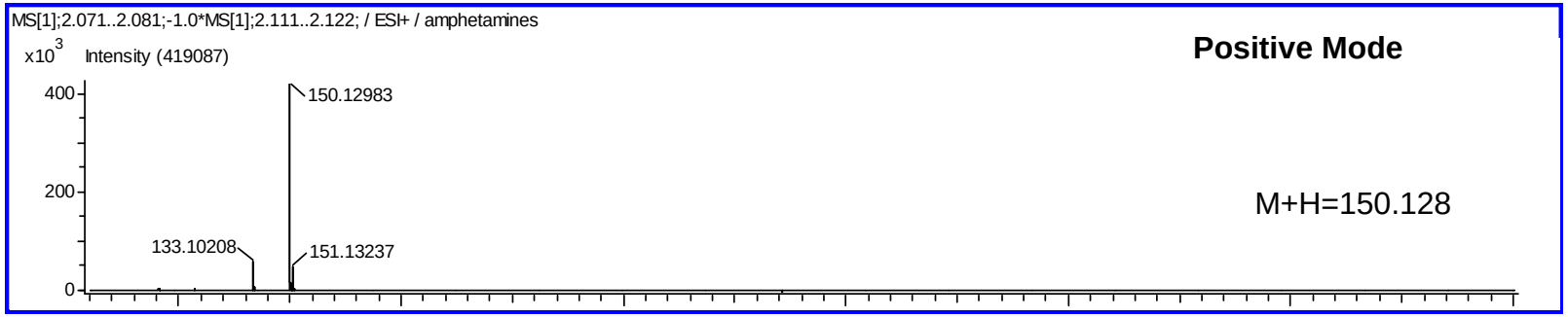


A-2. Phenethylamines (continued)

A-2.5 MDMA

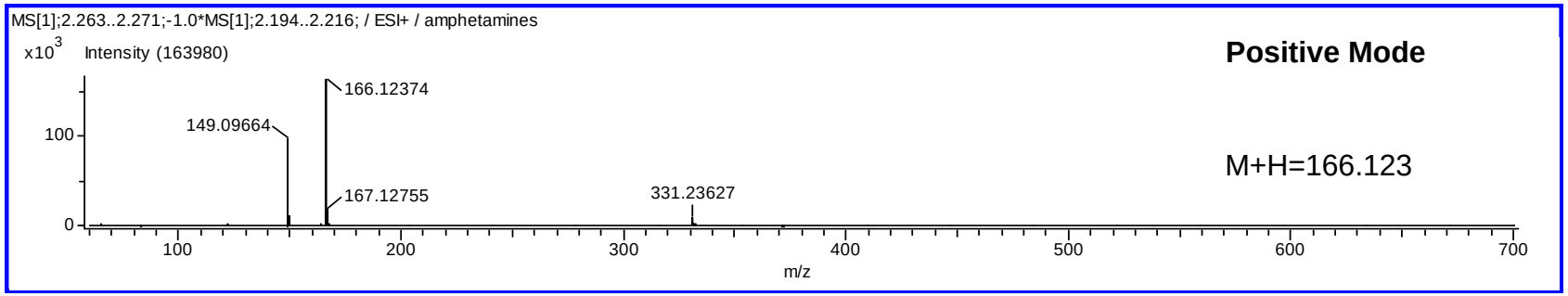


A-2.6. Phentermine

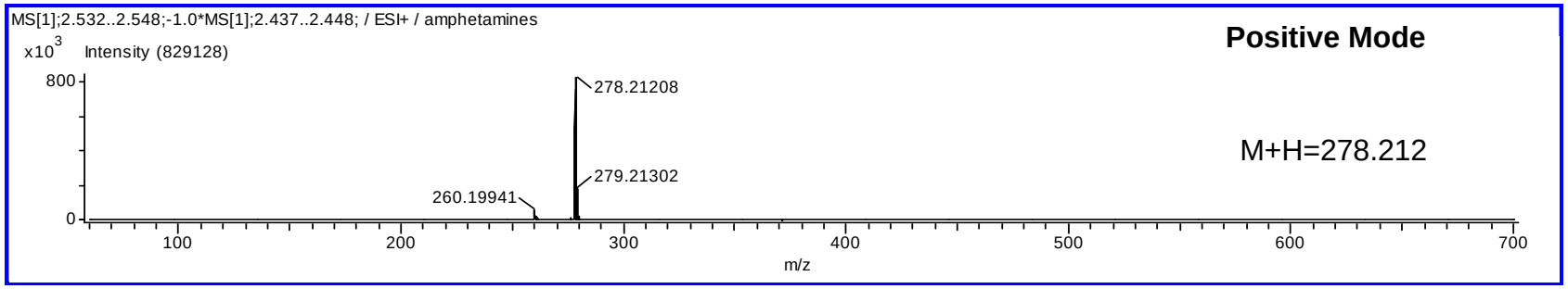


A-2. Phenethylamines (continued)

A-2.7. PMA

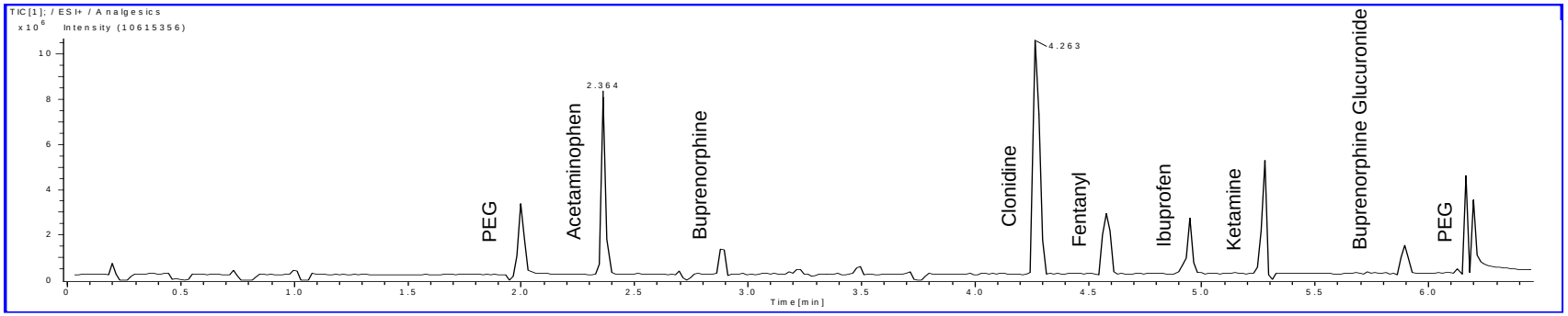


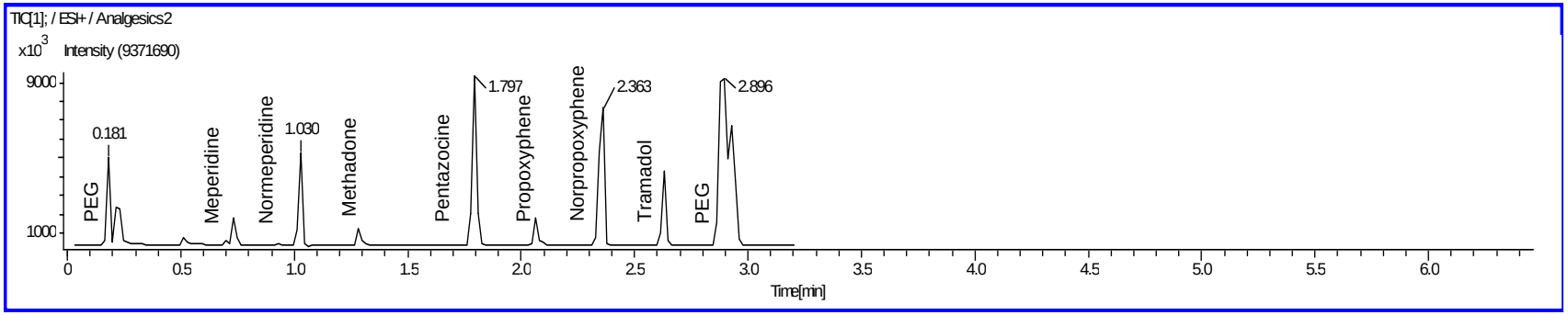
A-2.8. Venlafaxine

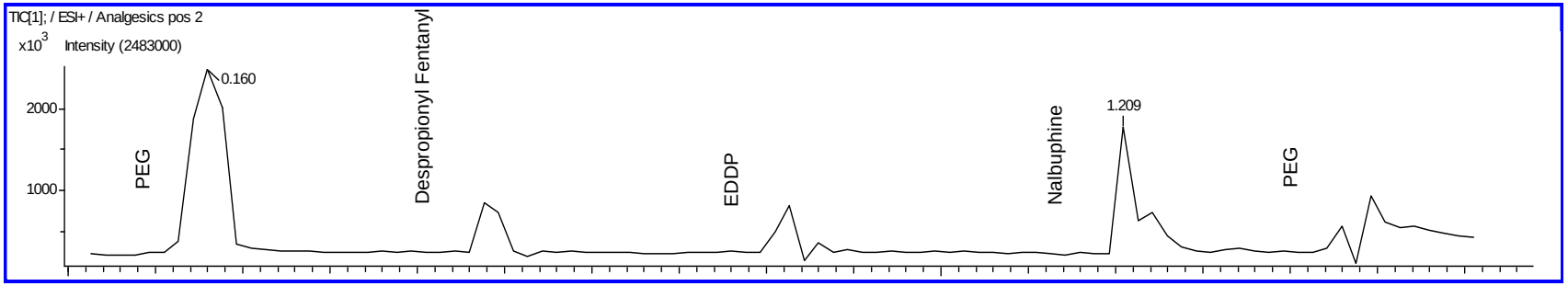


A-3. Analgesics (4/18/07)

Positive Mode

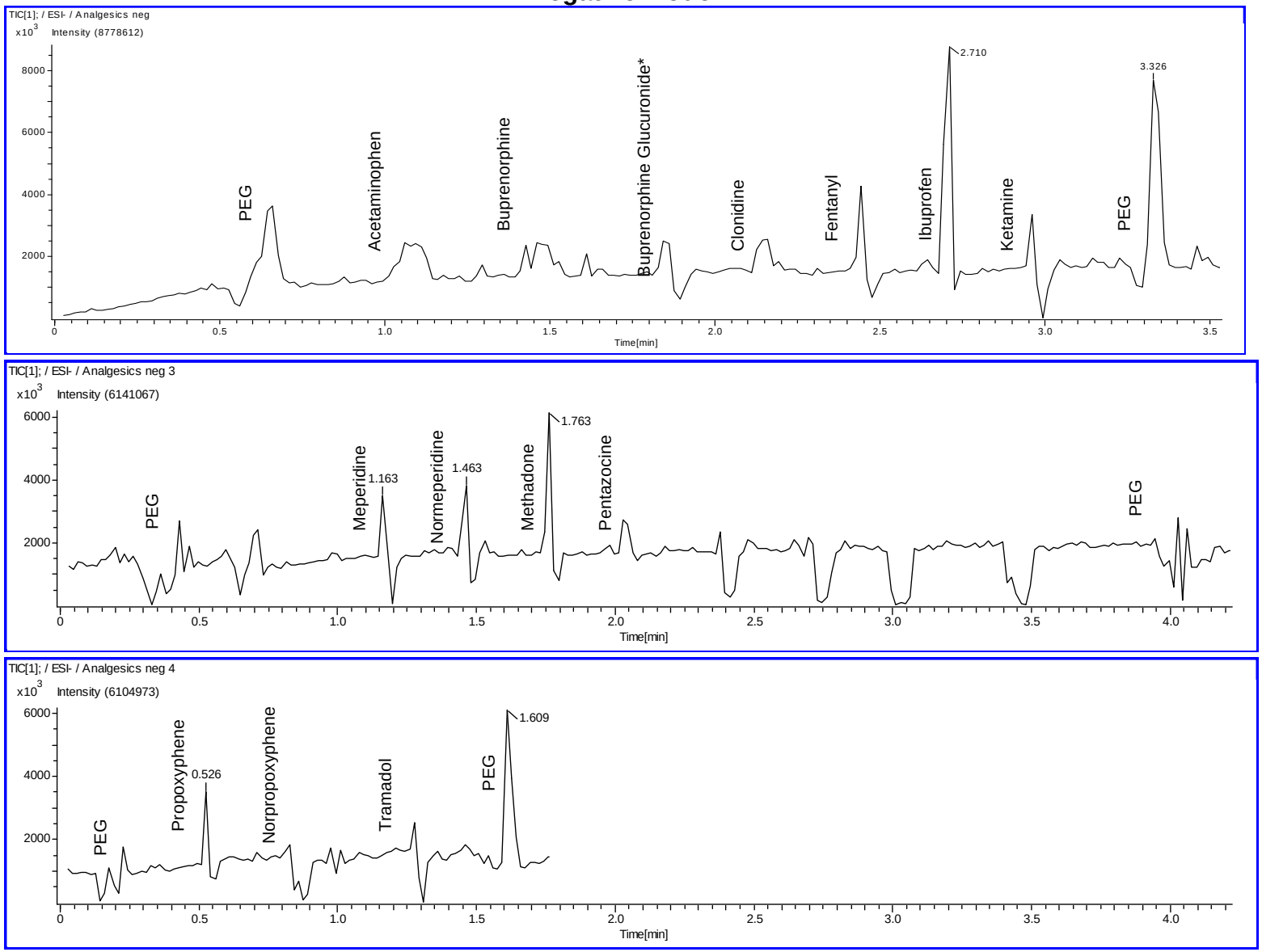






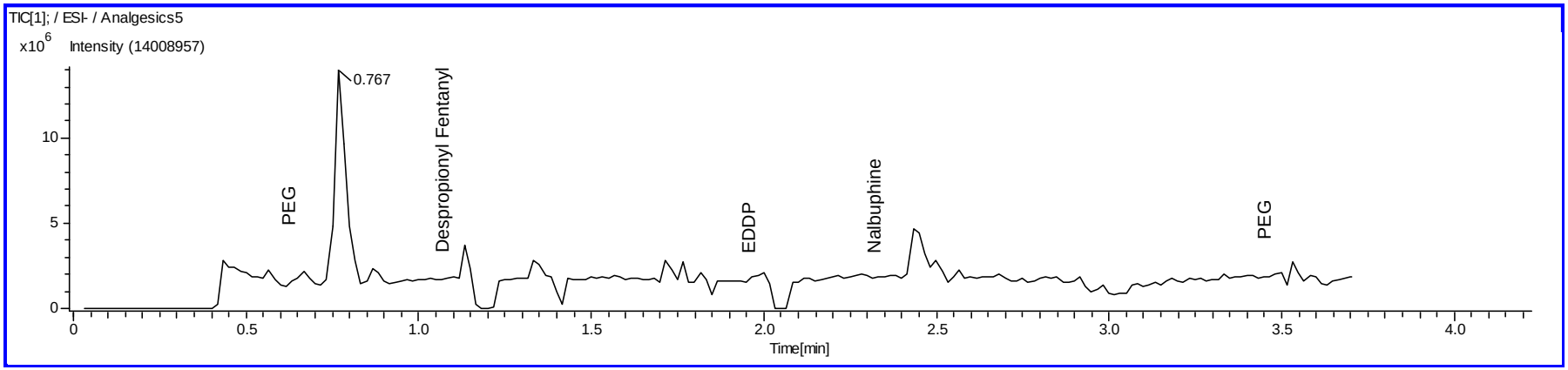
A-3. Analgesics (4/18/07) (continued)

Negative Mode



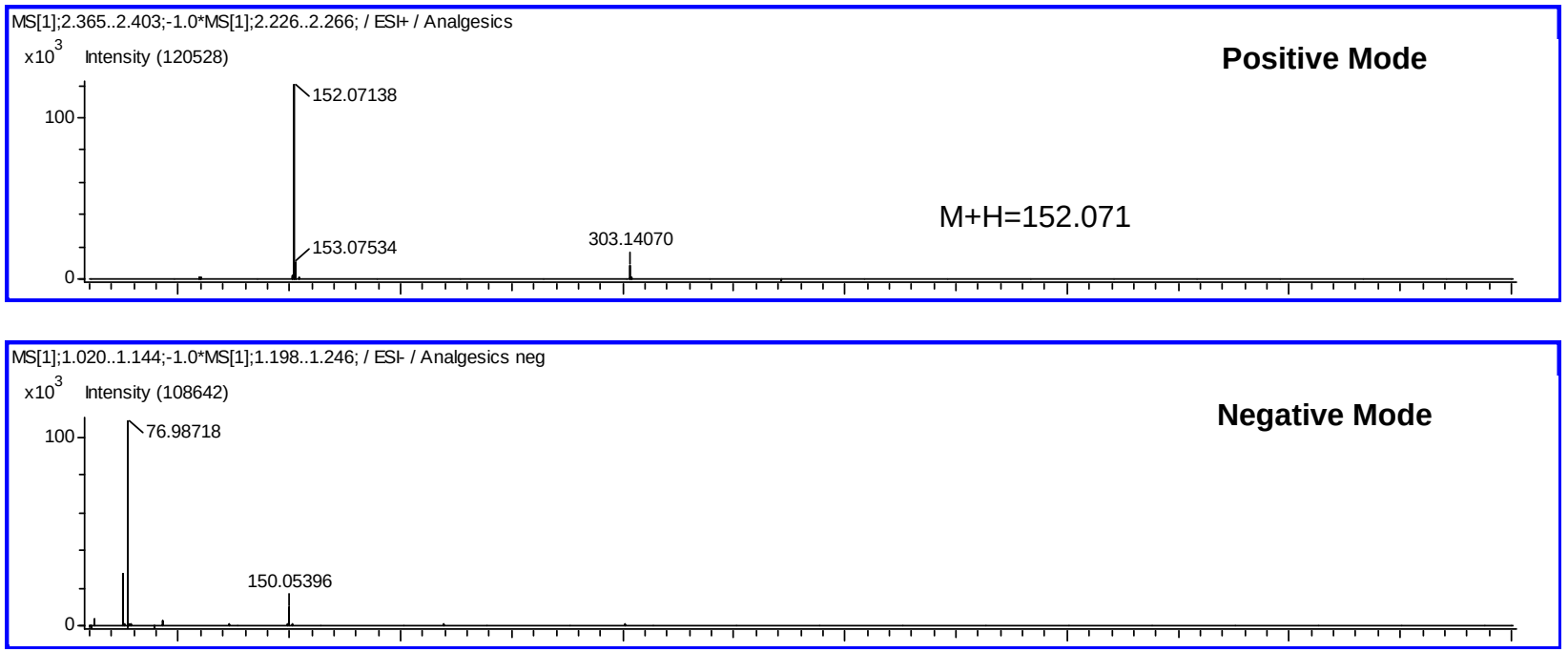
A-3. Analgesics (4/18/07) (continued)

Negative Mode



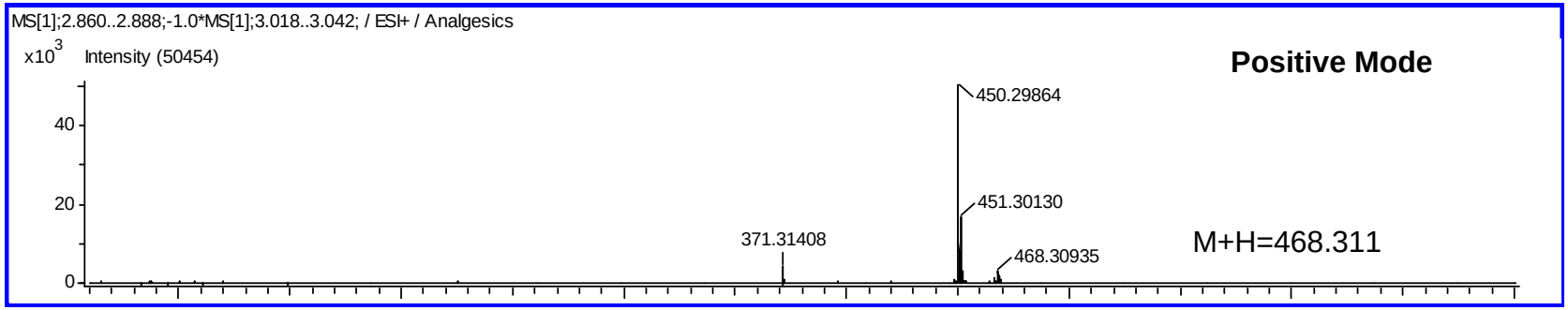
A-3. Analgesics (continued)

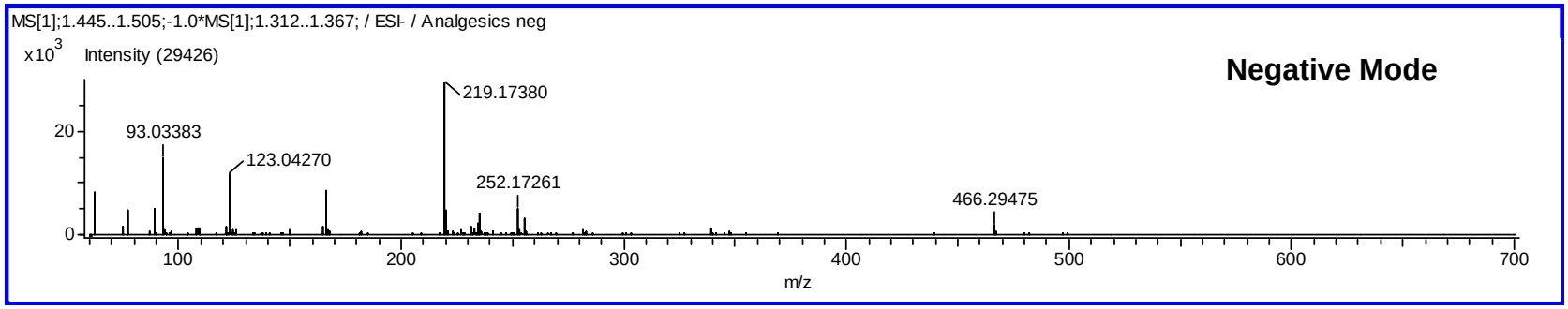
A-3.1. Acetaminophen



A-3. Analgesics (continued)

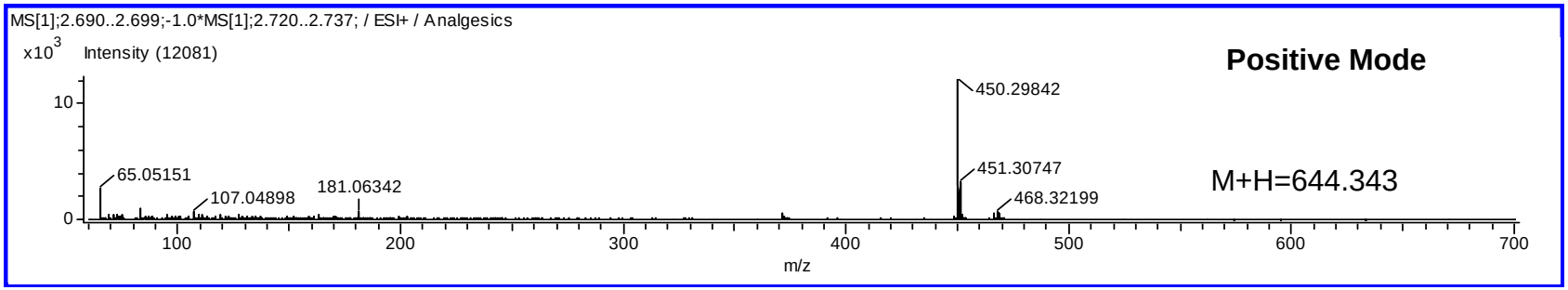
A-3.2. Buprenorphine

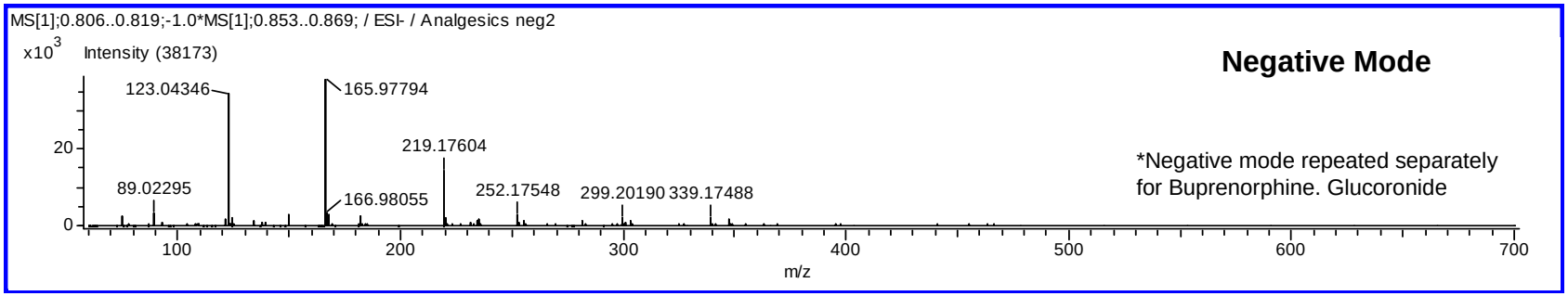




A-3. Analgesics (continued)

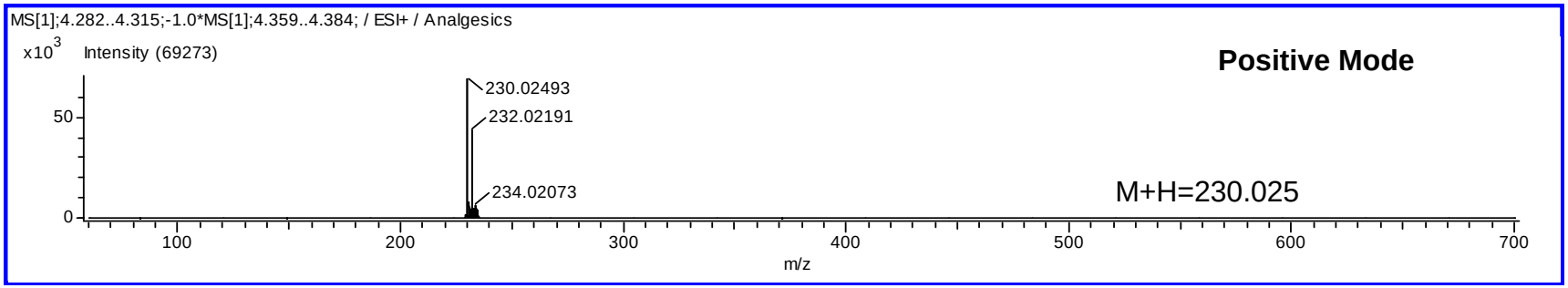
A-3.3. Buprenorphine Glucuronide

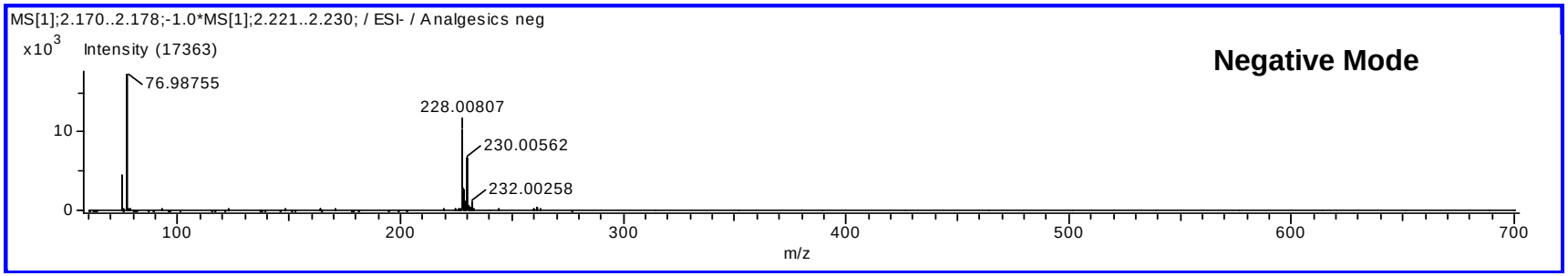




A-3. Analgesics (continued)

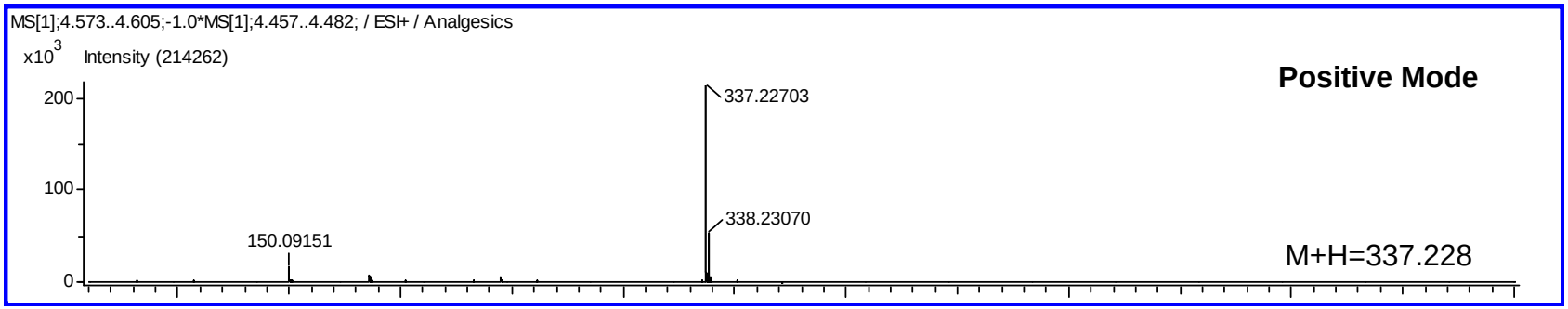
A-3.4. Clonidine

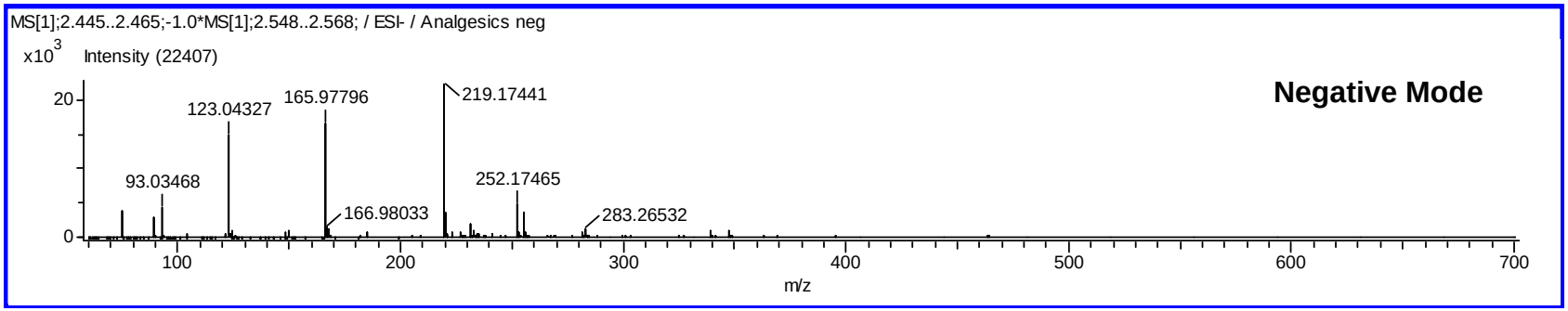




A-3. Analgesics (continued)

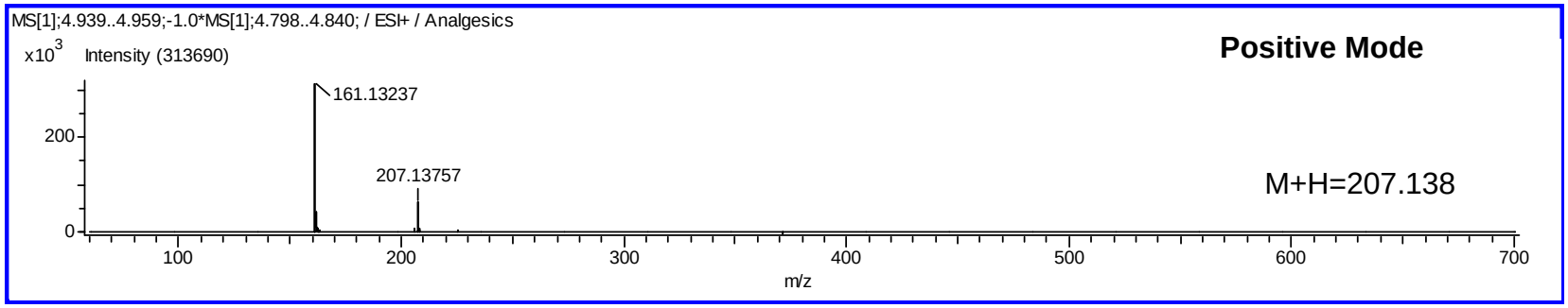
A-3.5. Fentanyl

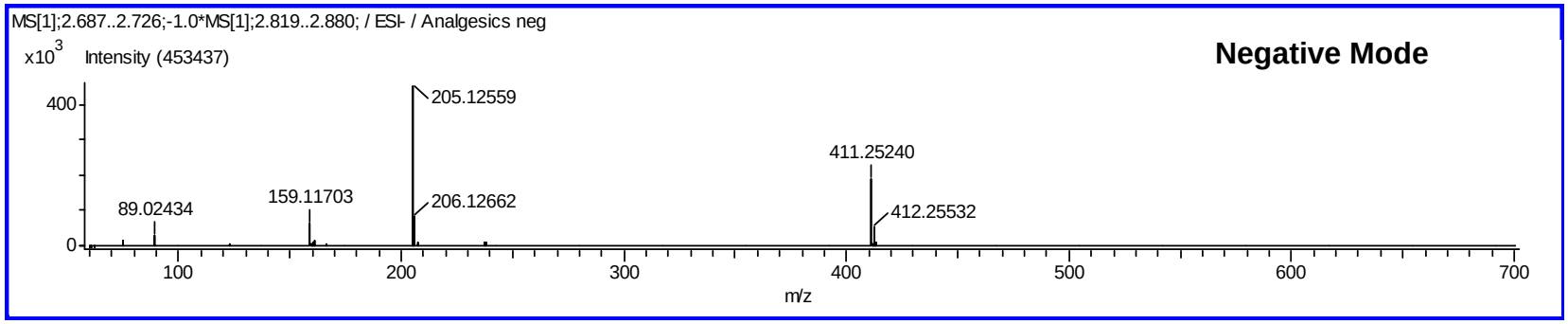




A-3. Analgesics (continued)

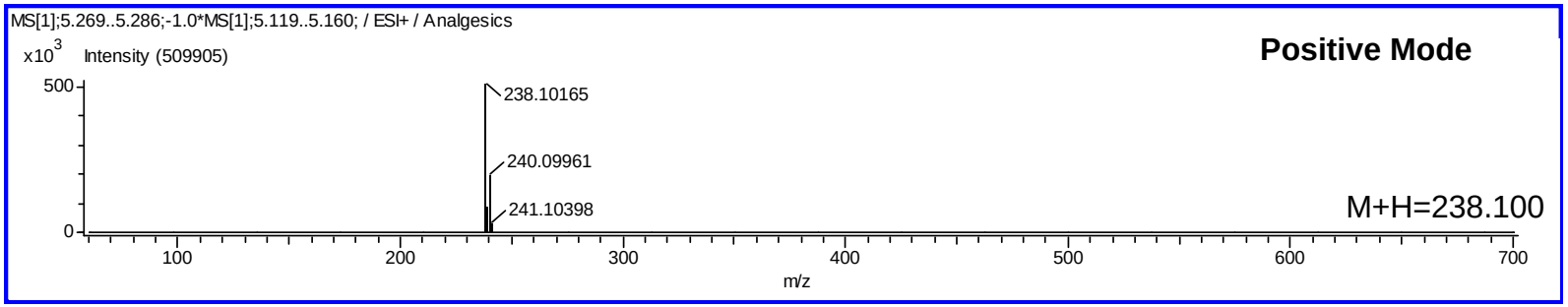
A-3.6. Ibuprofen

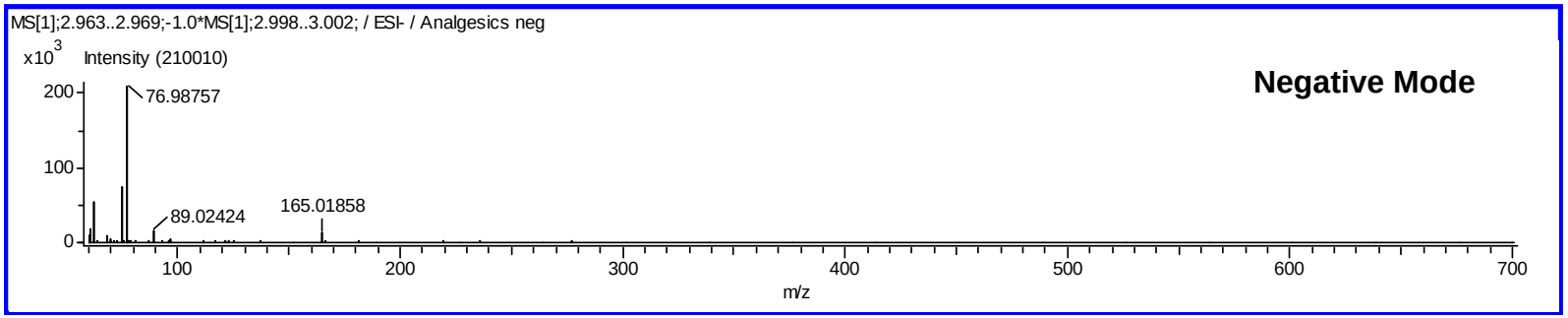




A-3. Analgesics (continued)

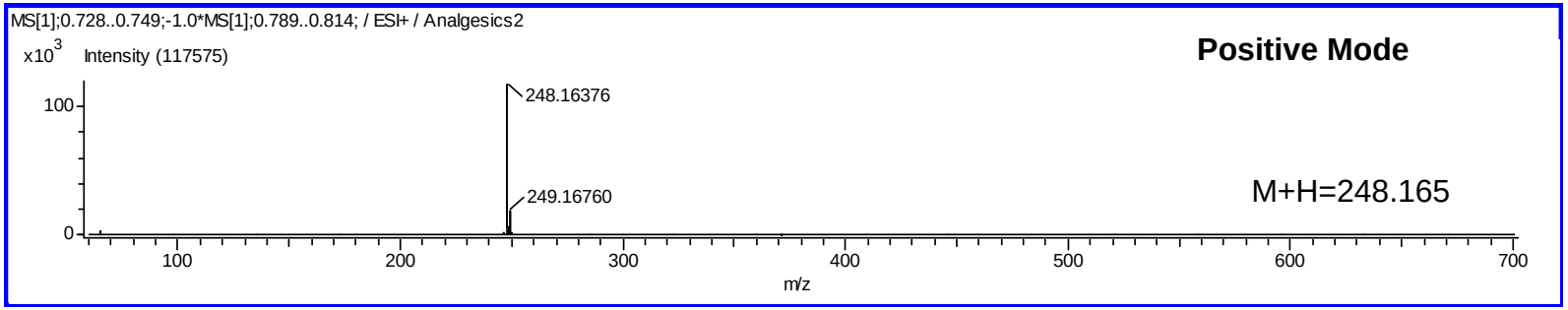
A-3.7. Ketamine

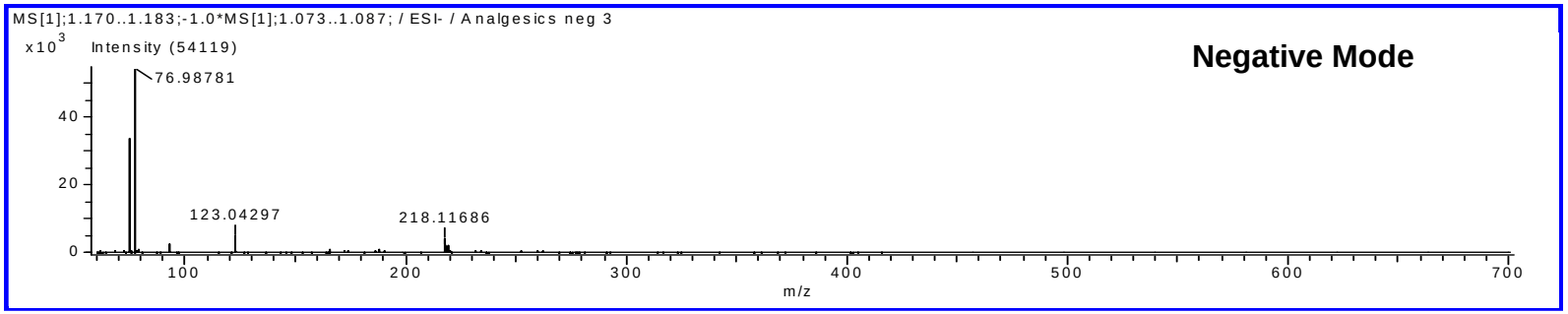




A-3. Analgesics (continued)

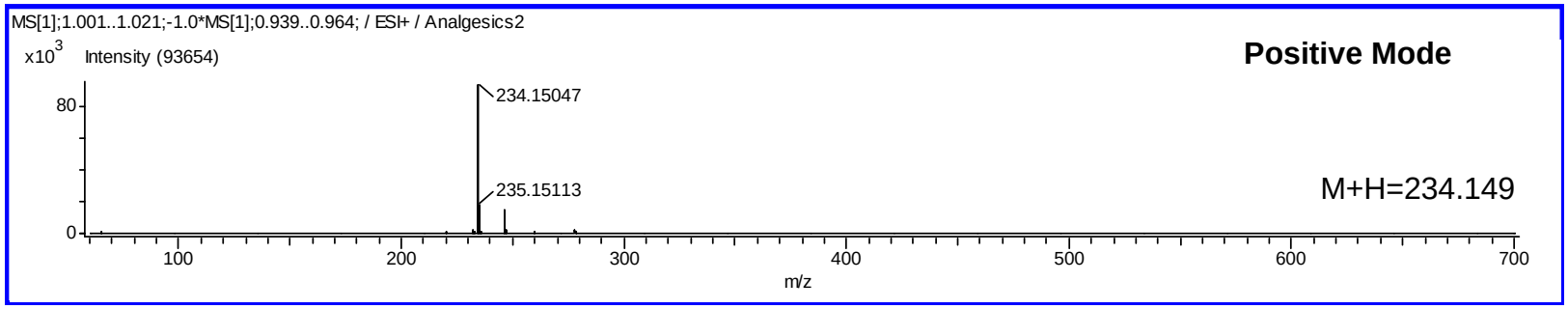
A-3.8. Meperidine

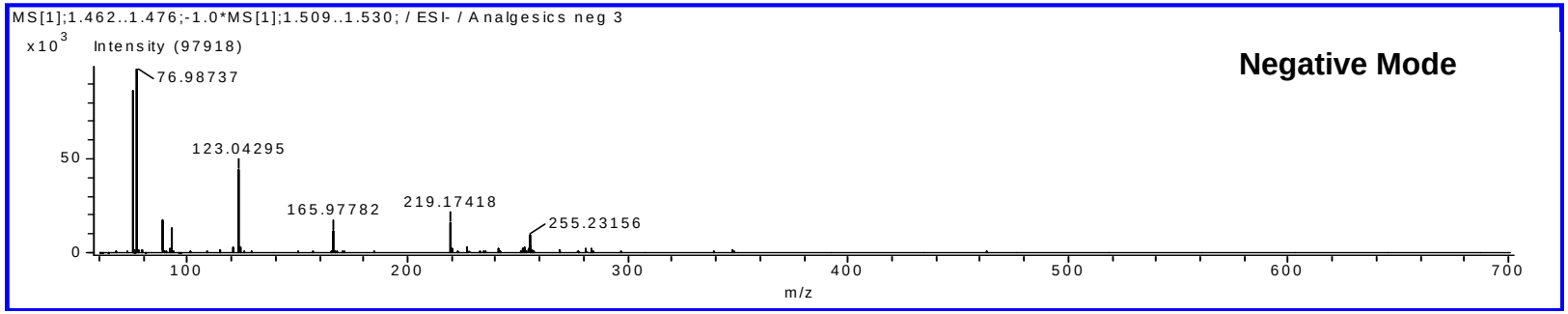




A-3. Analgesics (continued)

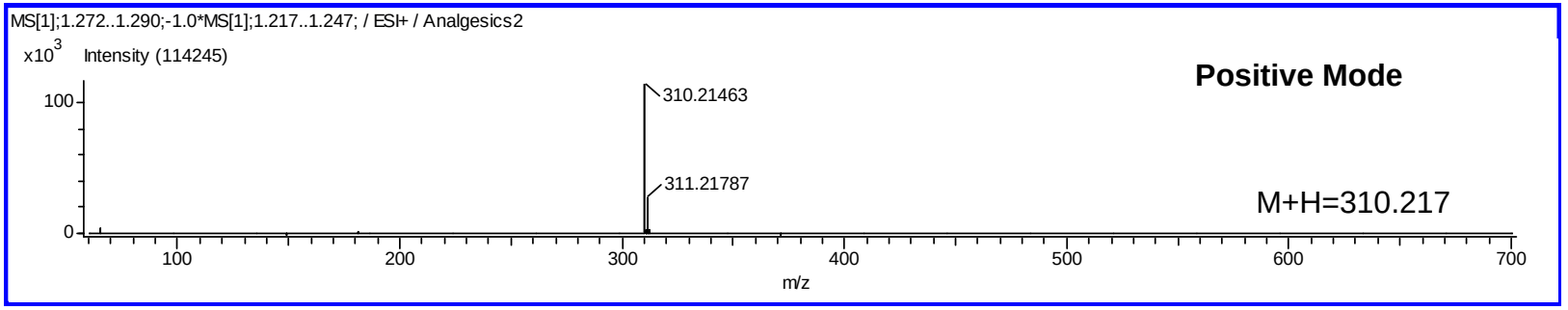
A-3.9. Normeperidine

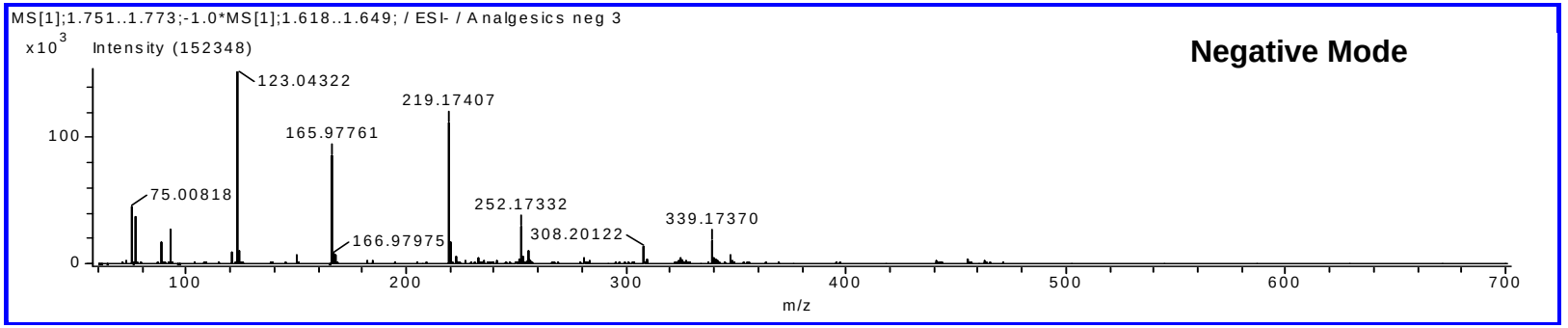




A-3. Analgesics (continued)

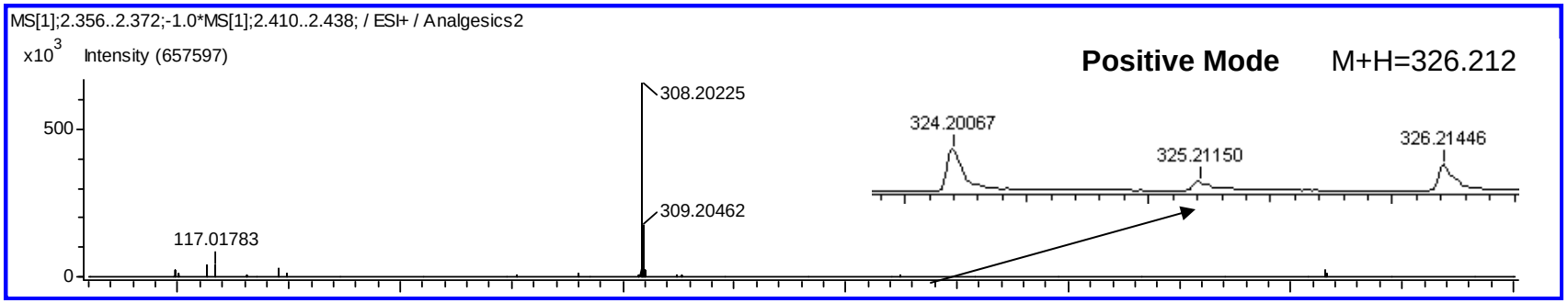
A-3.10. Methadone

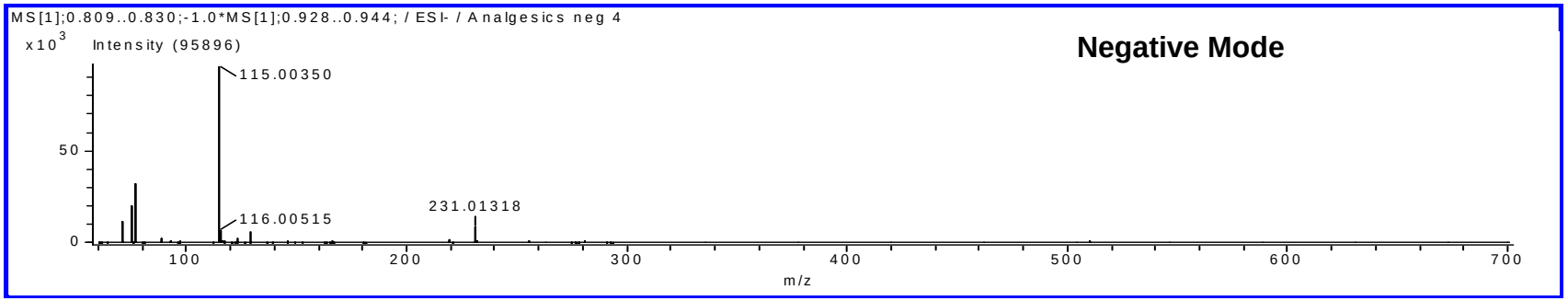




A-3. Analgesics (continued)

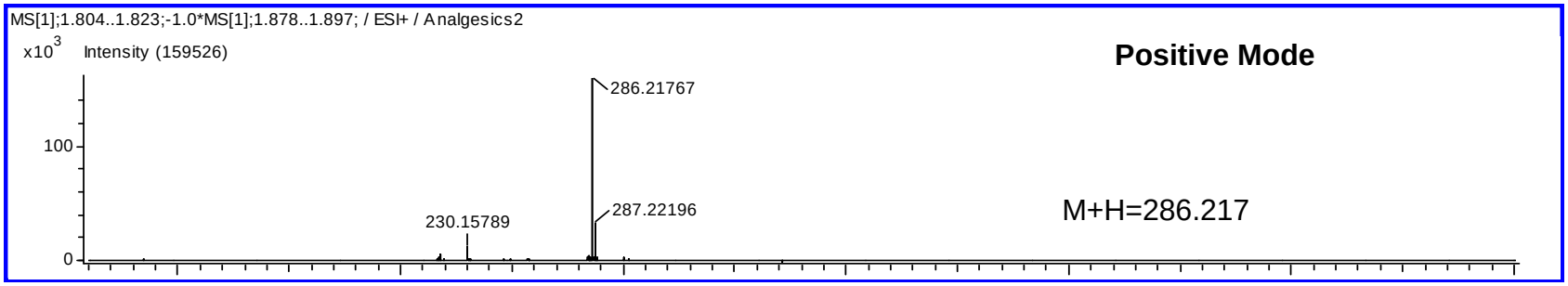
A-3.11. Norpropoxyphene

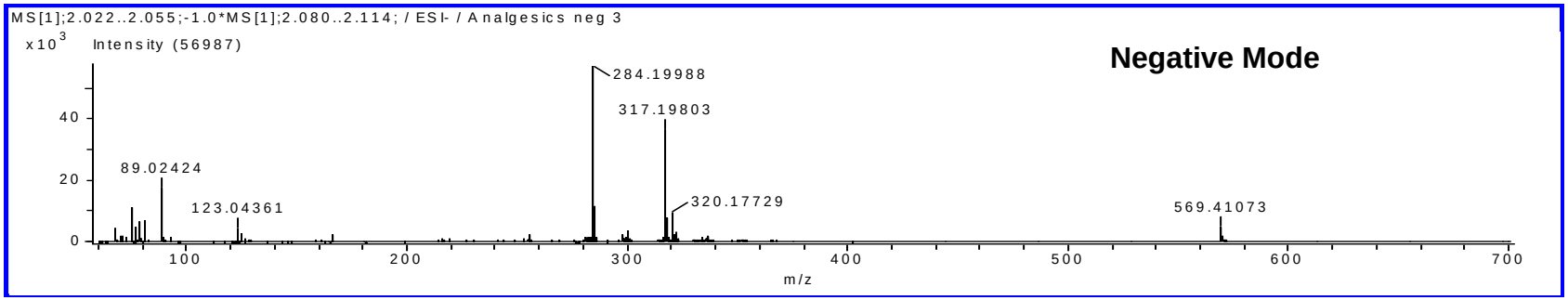




A-3. Analgesics (continued)

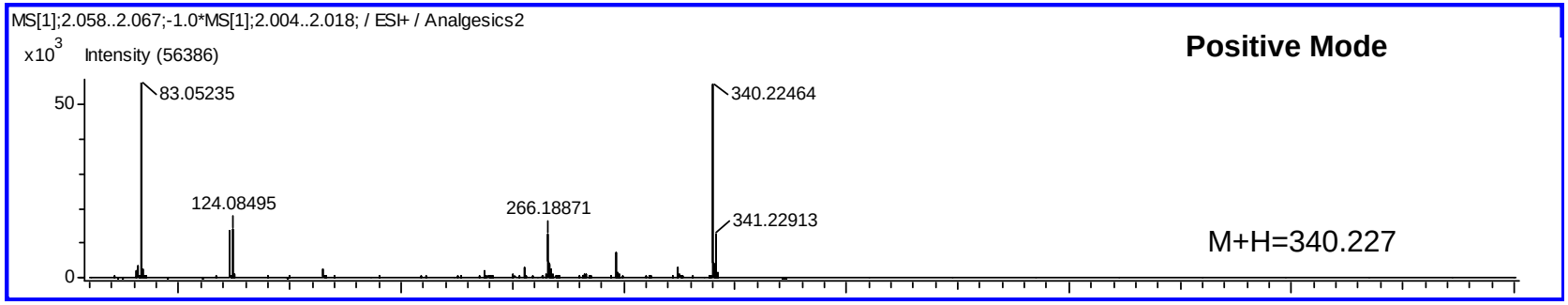
A-3.12. Pentazocine

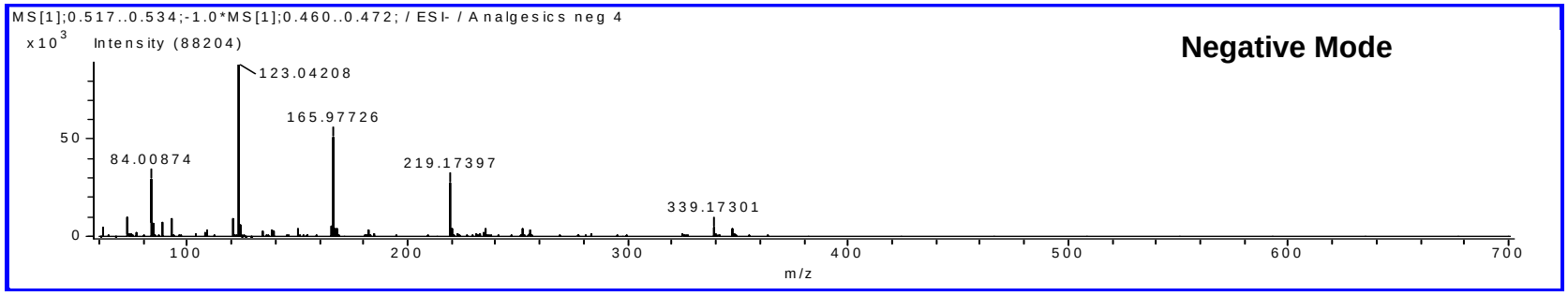




A-3. Analgesics (continued)

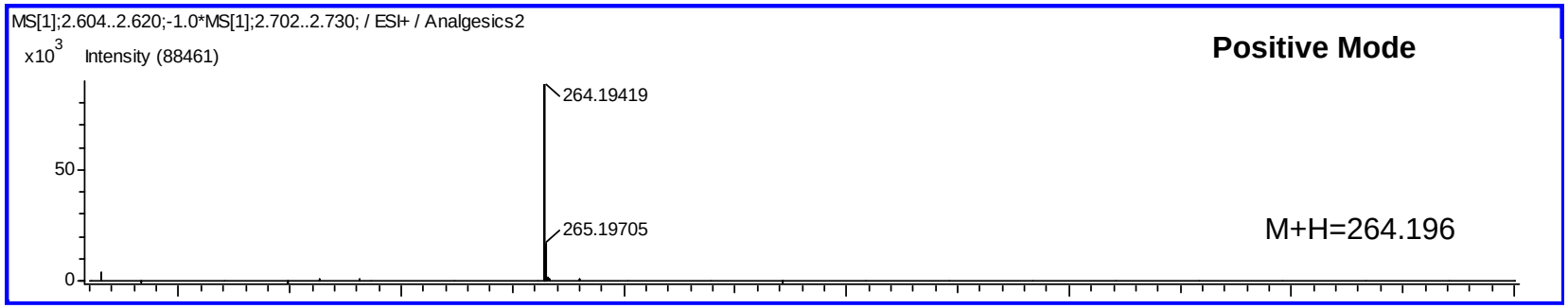
A-3.13. Propoxyphene

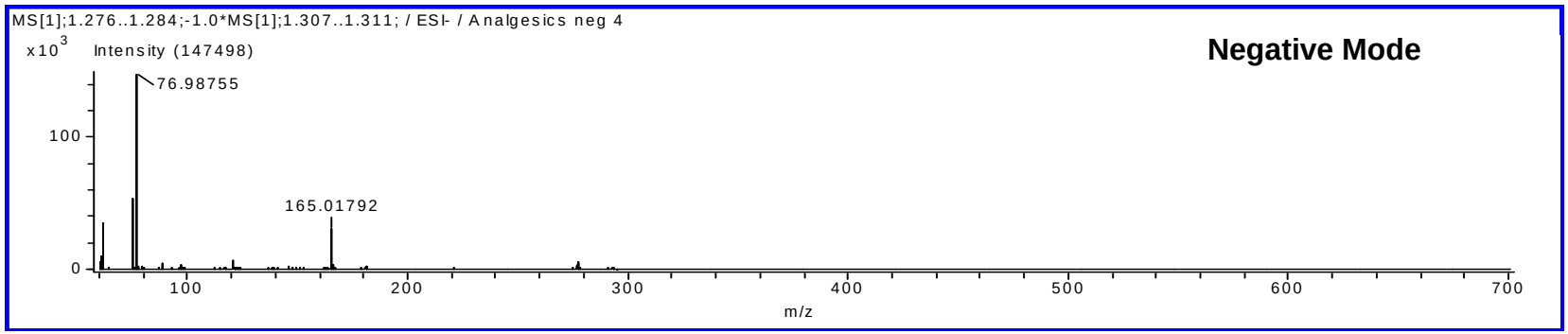




A-3. Analgesics (continued)

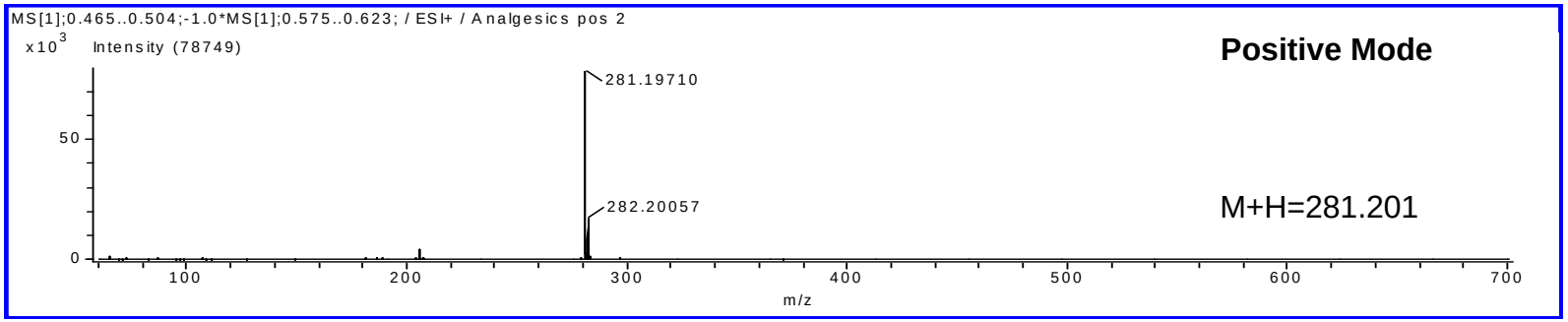
A-3.14. cis-Tramadol

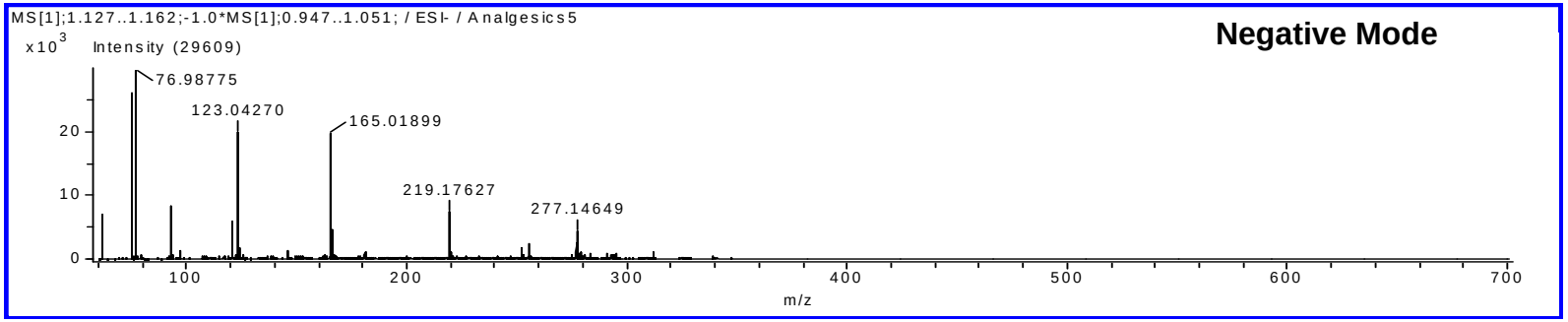




A-3. Analgesics (continued)

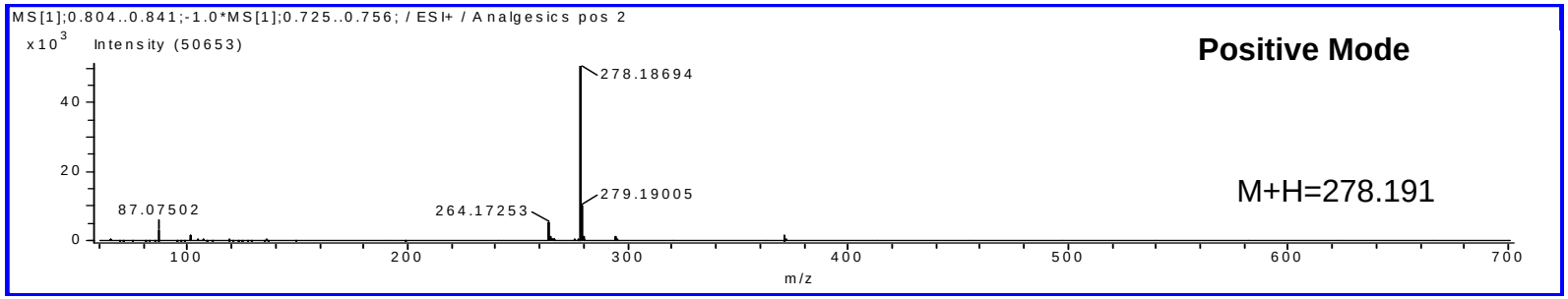
A-3.15. Despropionyl fentanyl

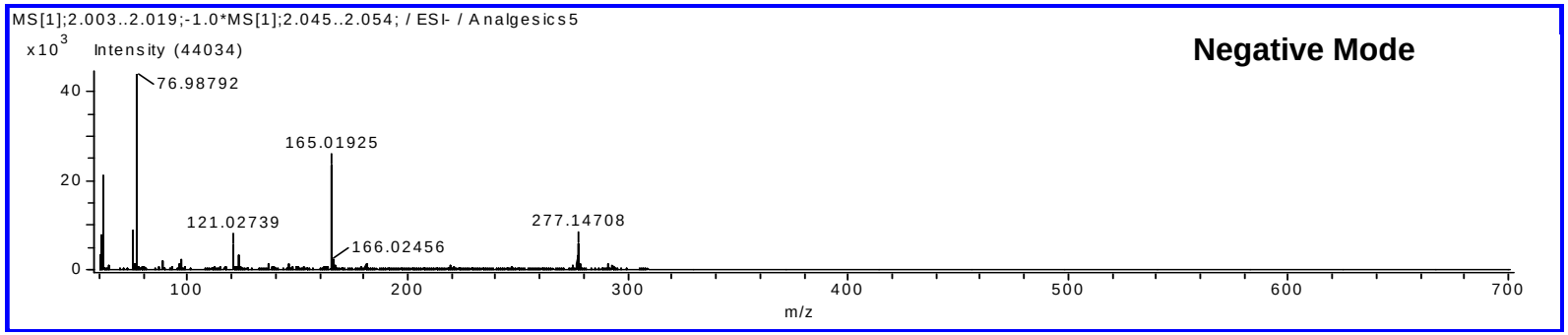




A-3. Analgesics (continued)

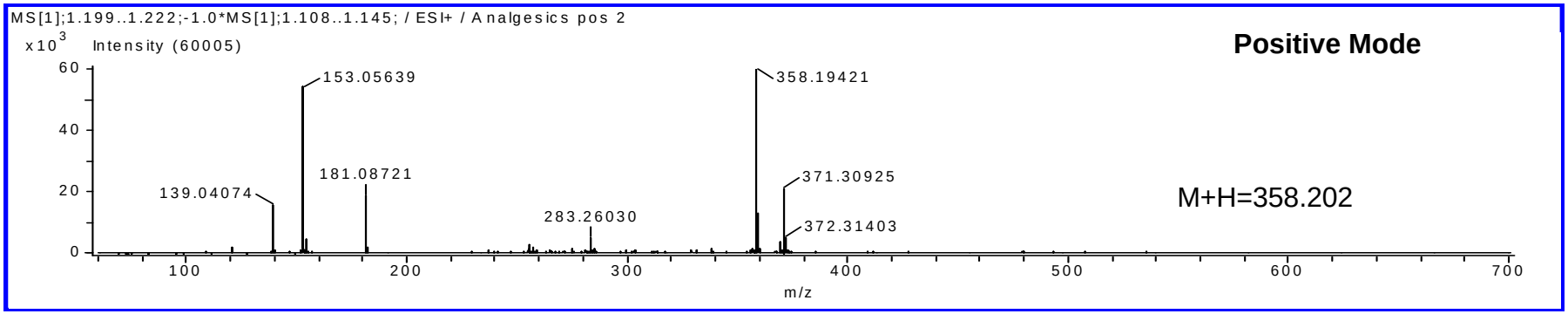
A-3.16. EDDP

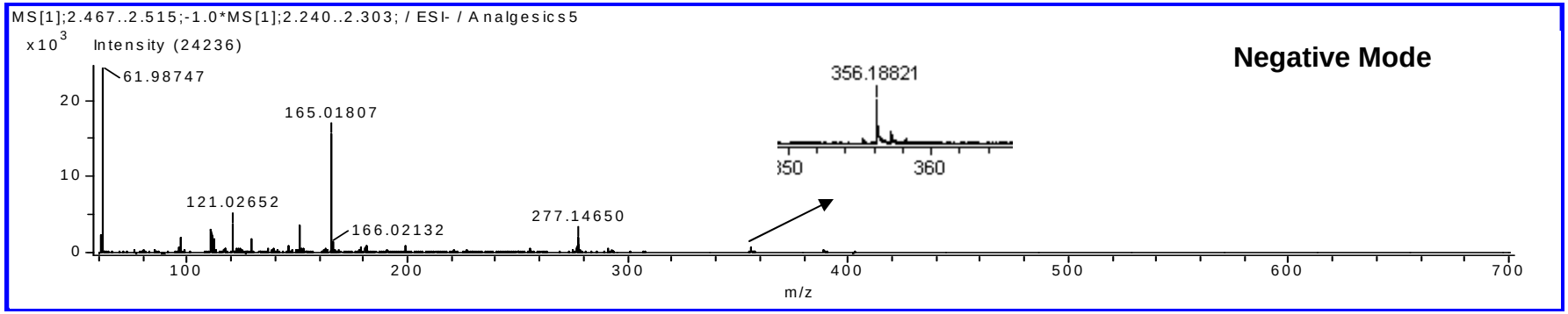




A-3. Analgesics (continued)

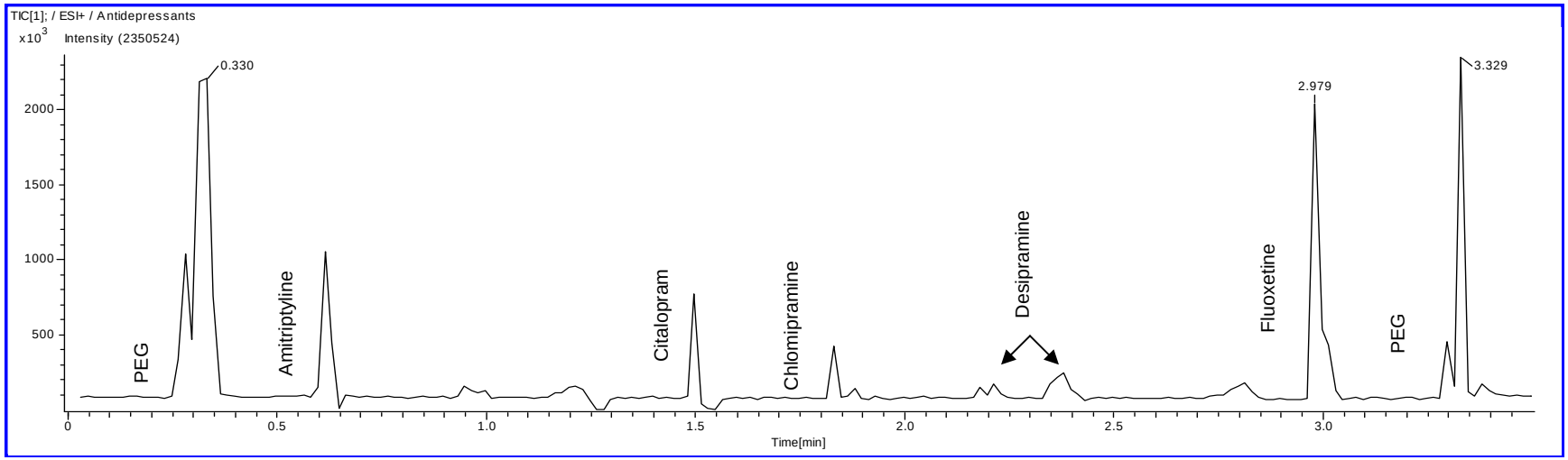
A-3.17. Nalbuphine

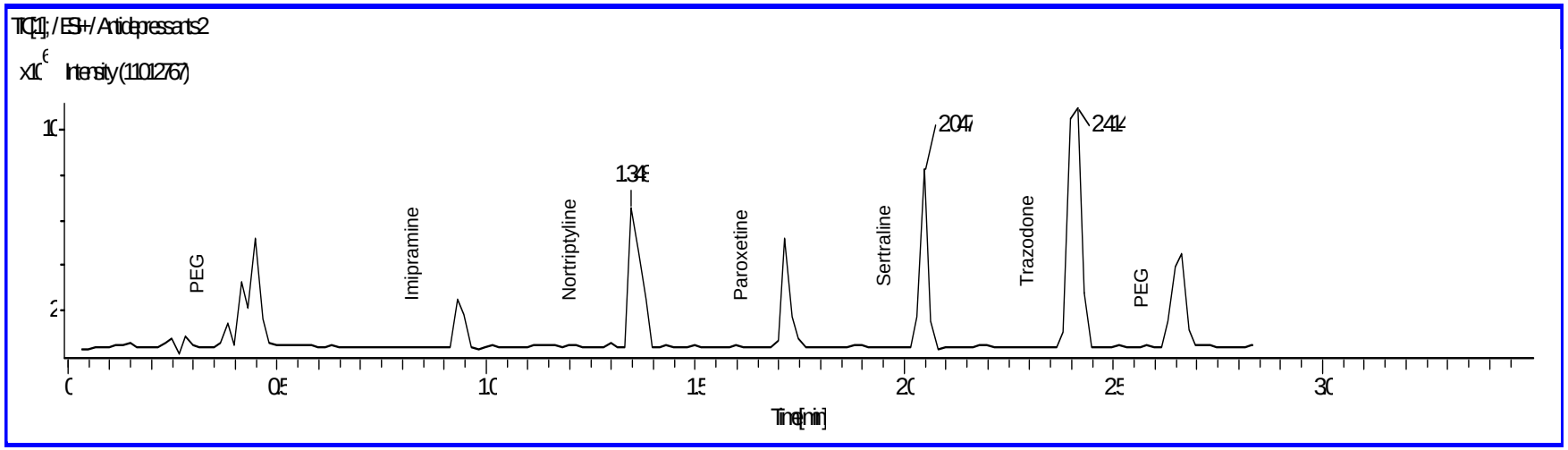




A-4. Antidepressants (4/7/07)

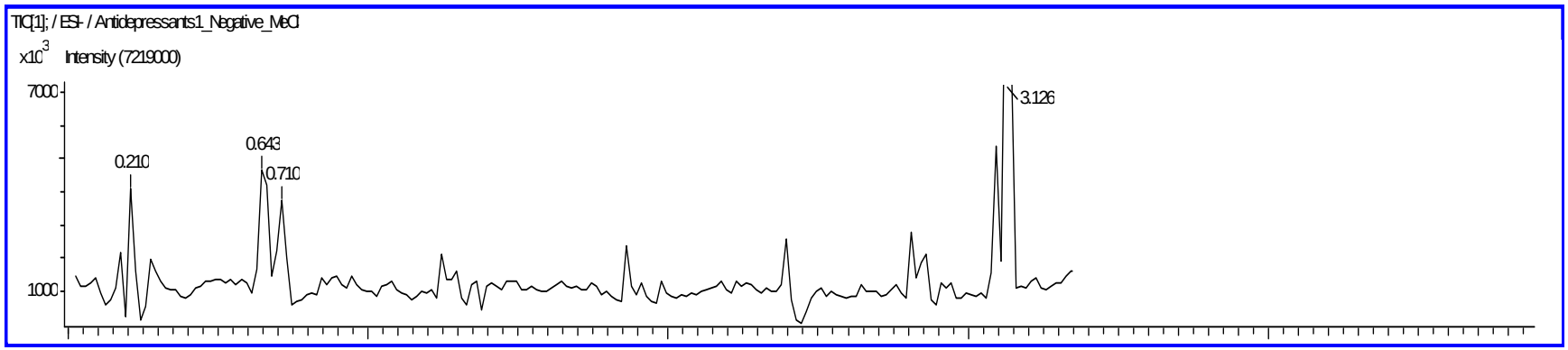
Positive Mode





A-4. Antidepressants (4/7/07) (continued)

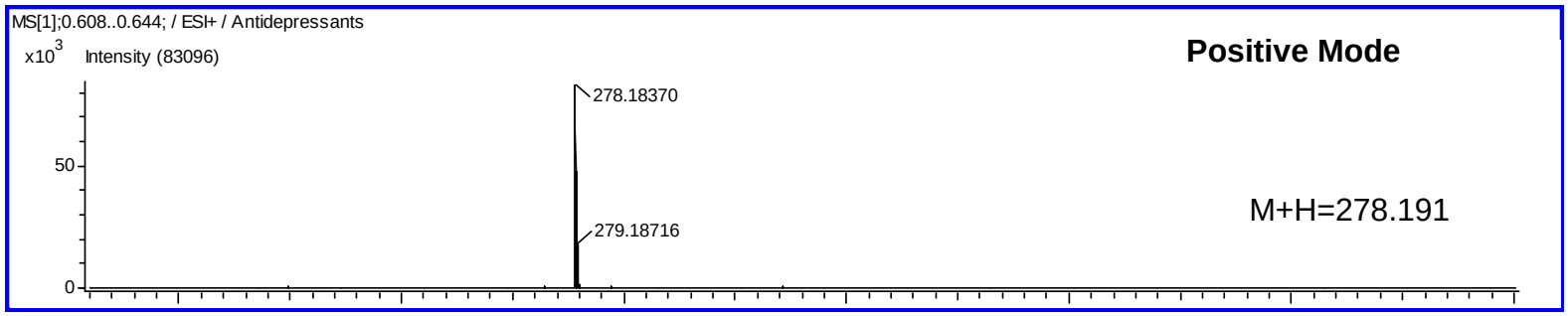
Negative Mode



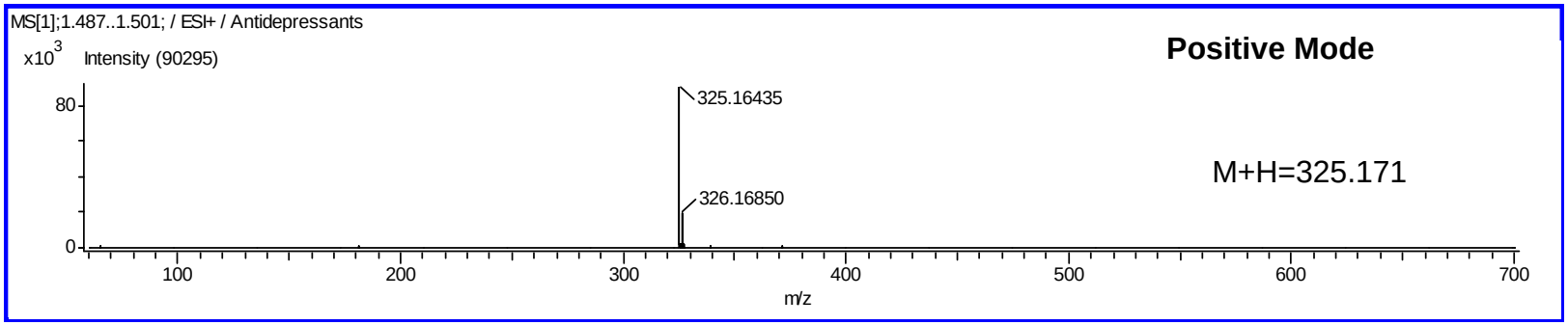
Spectra not applicable

A-4. Antidepressants (continued)

A-4.1. Amitriptyline

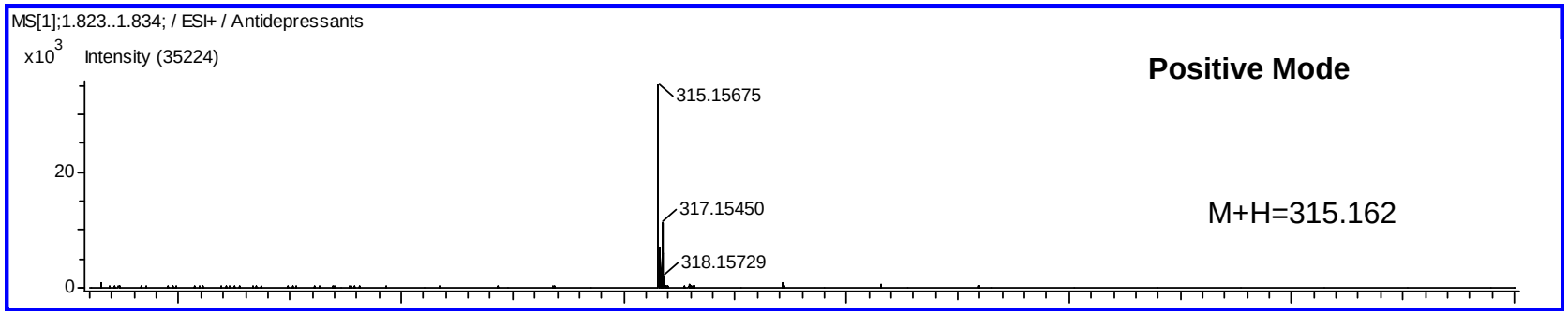


A-4.2. Citalopram

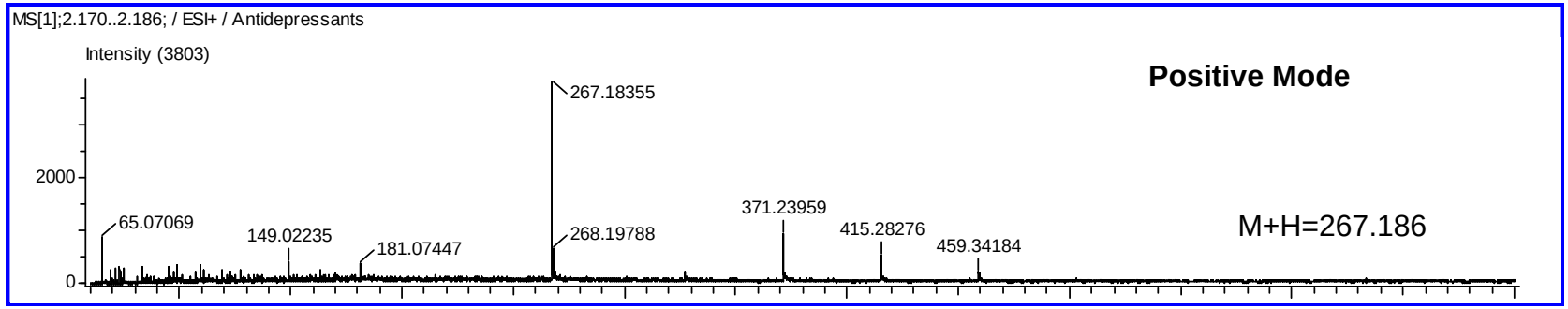


A-4. Antidepressants (continued)

A-4.3. Chlomipramine

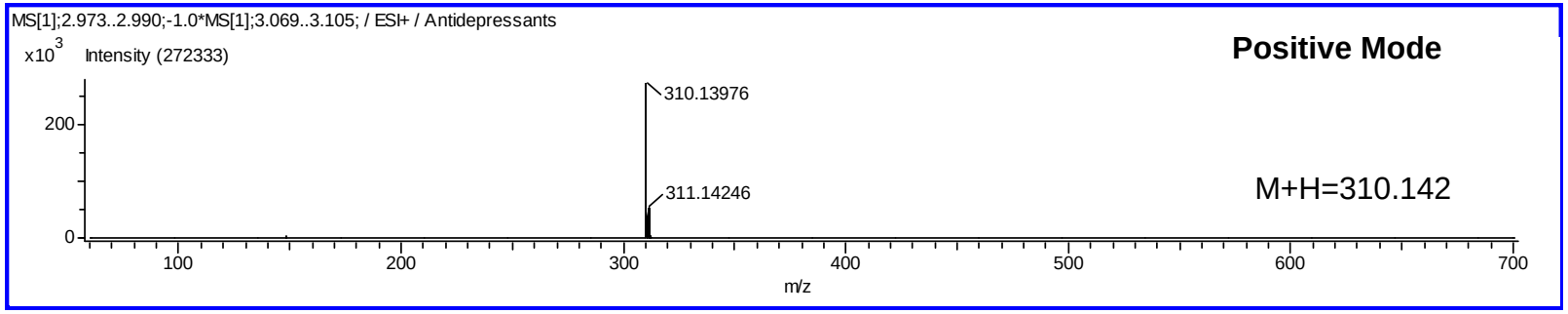


A-4.4. Desipramine

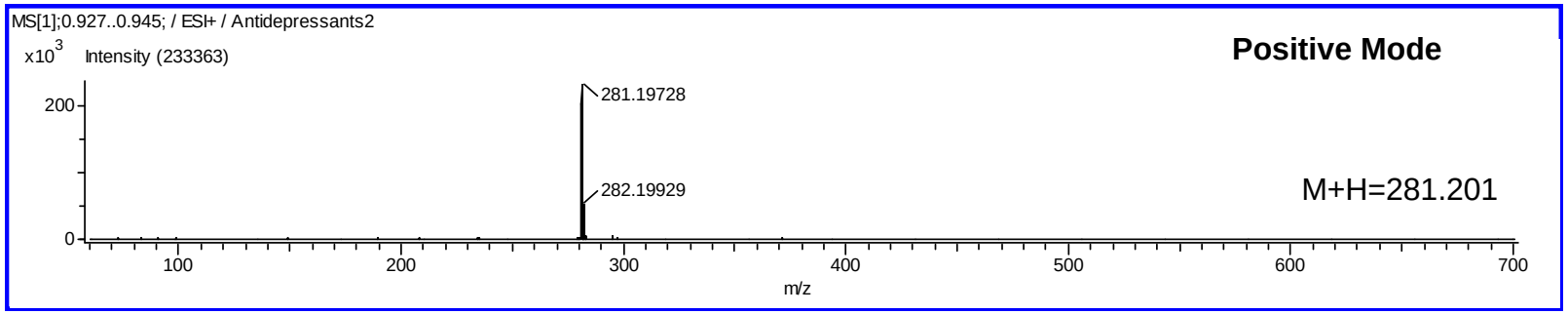


A-4. Antidepressants (continued)

A-4.5. Fluoxetine

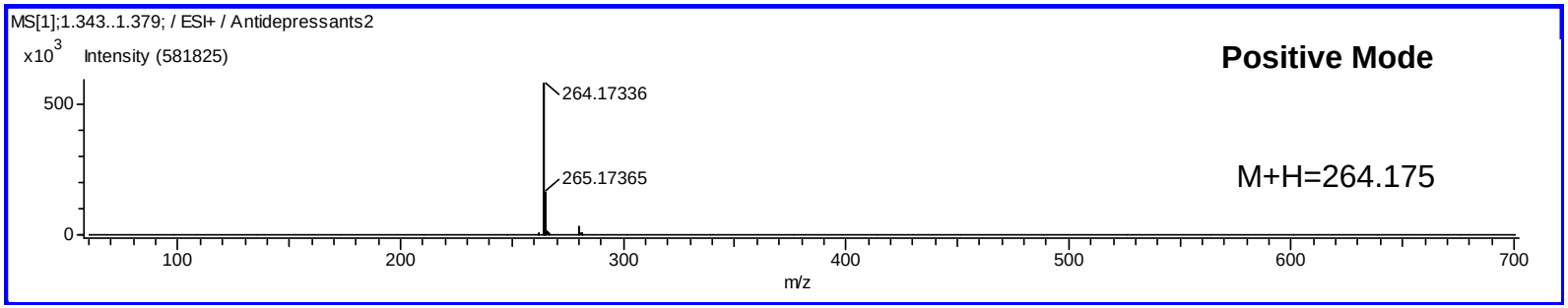


A-4.6. Imipramine

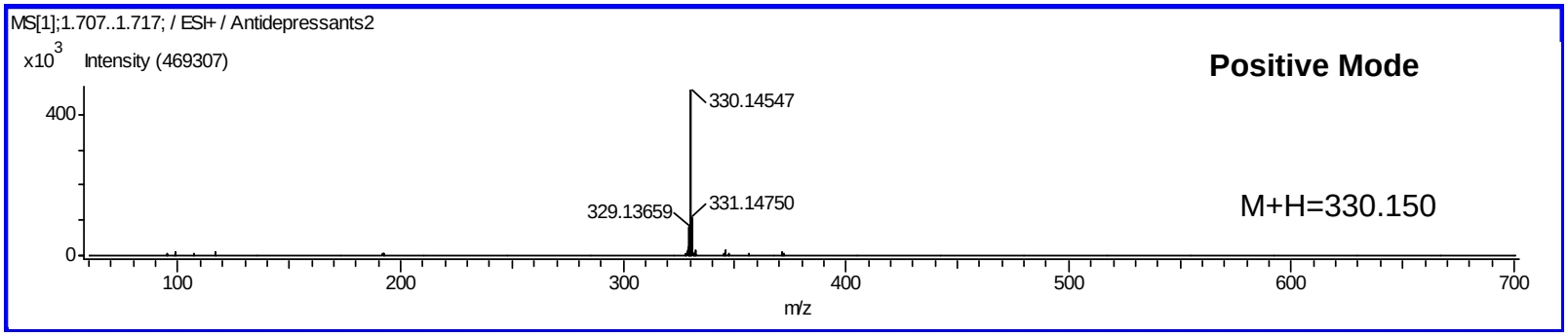


A-4. Antidepressants (continued)

A-4.7. Nortriptyline

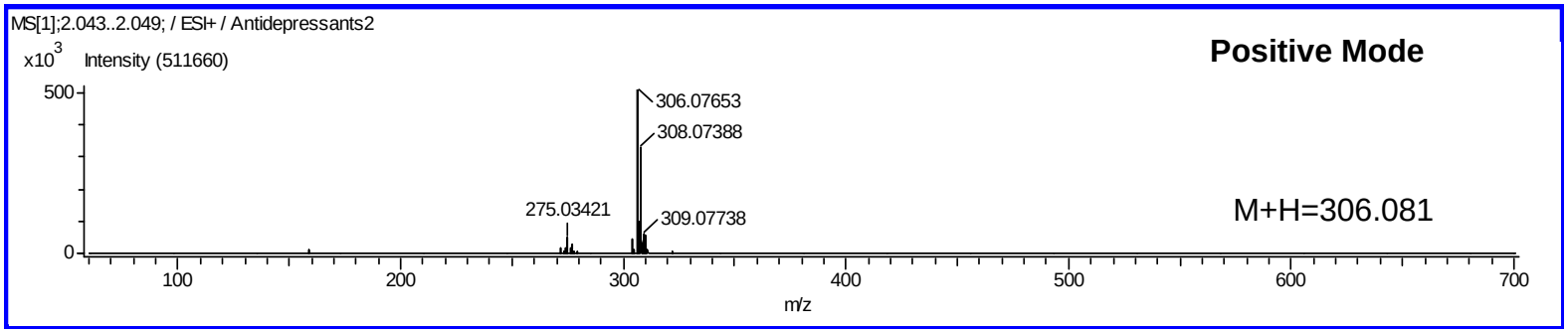


A-4.8. Paroxetine

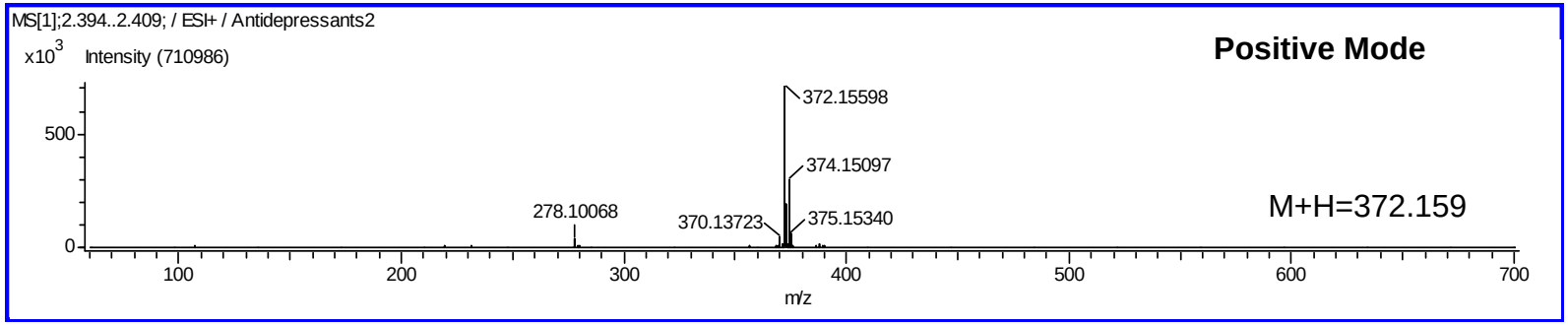


A-4. Antidepressants (continued)

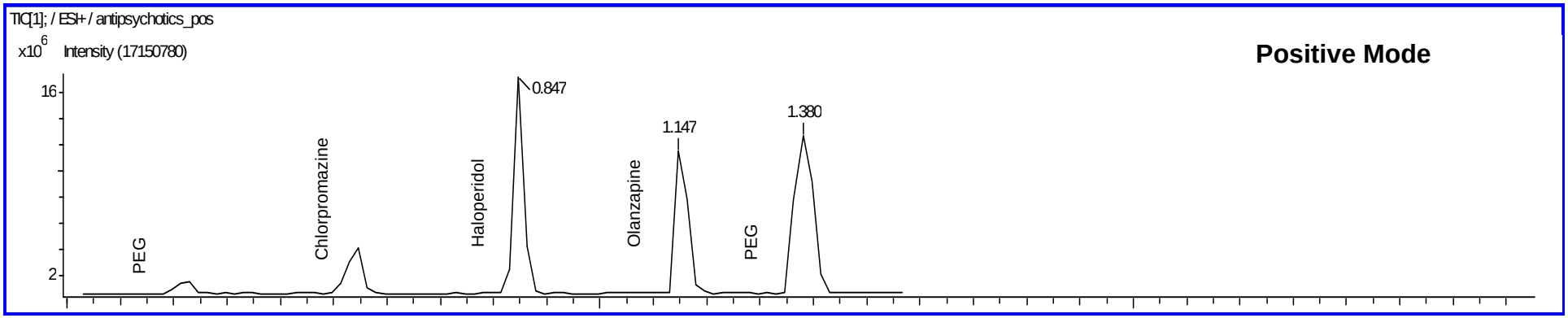
A-4.9. Sertraline

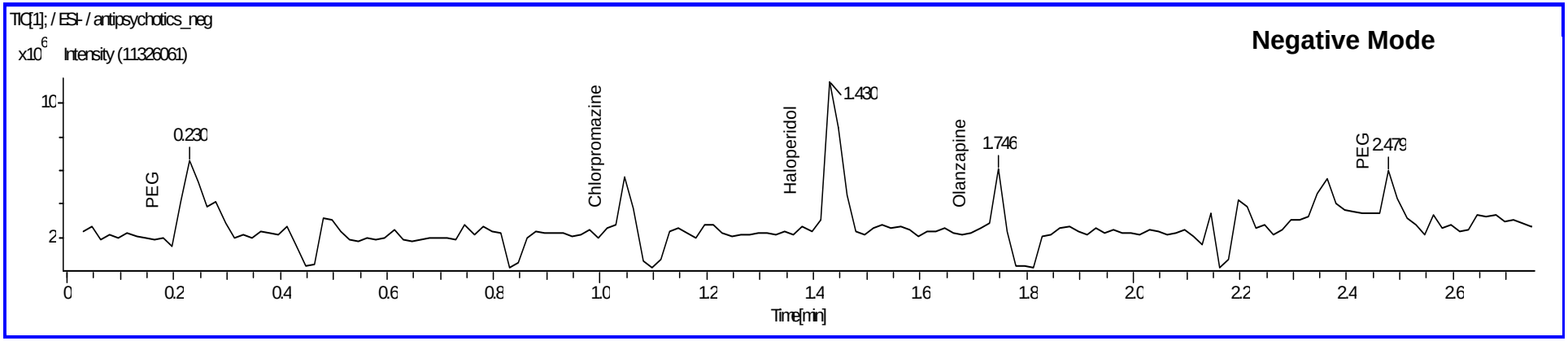


A-4.10. Trazodone



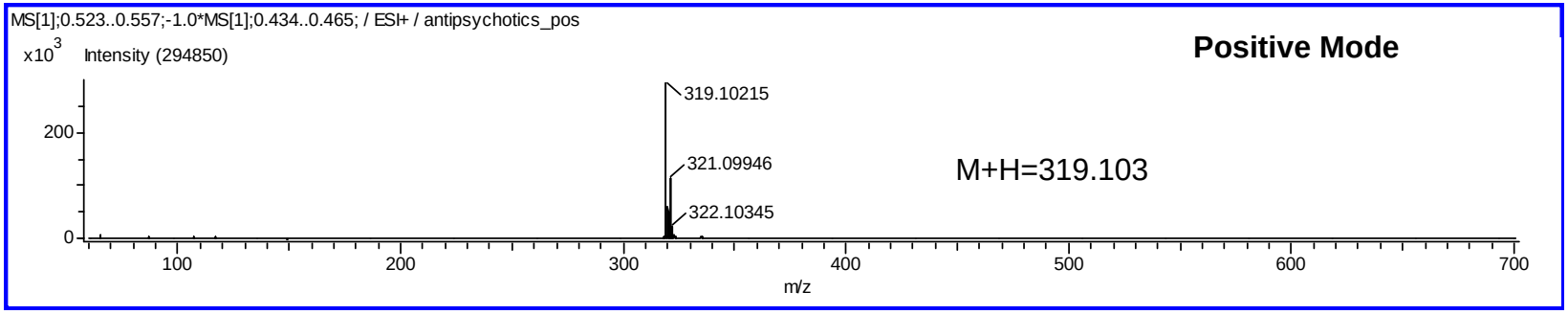
A-5. Antipsychotics (4/17/07)

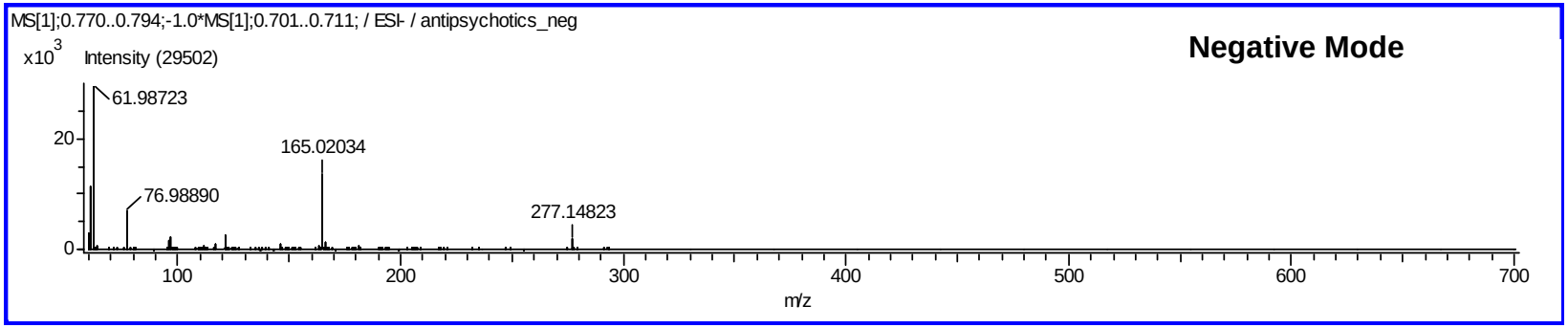




A-5. Antipsychotics (continued)

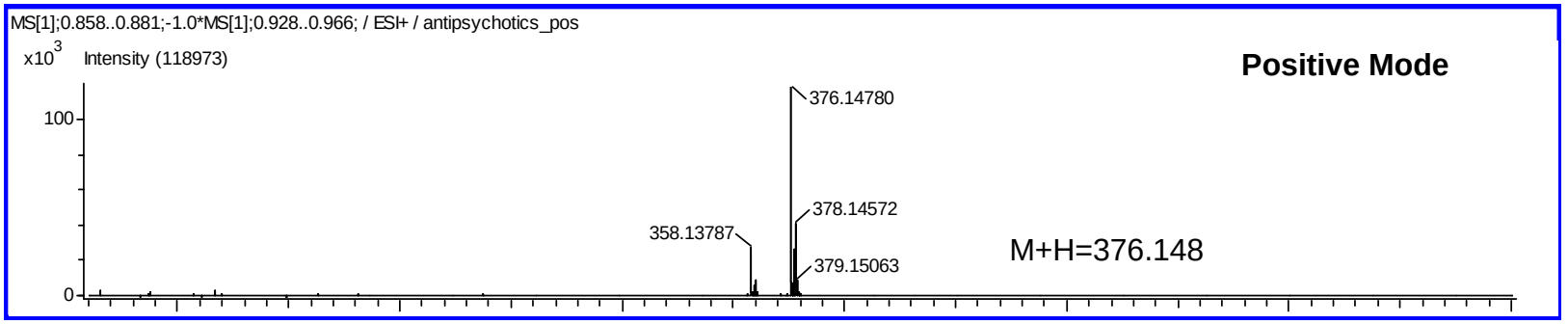
A-5.1. Chlorpromazine

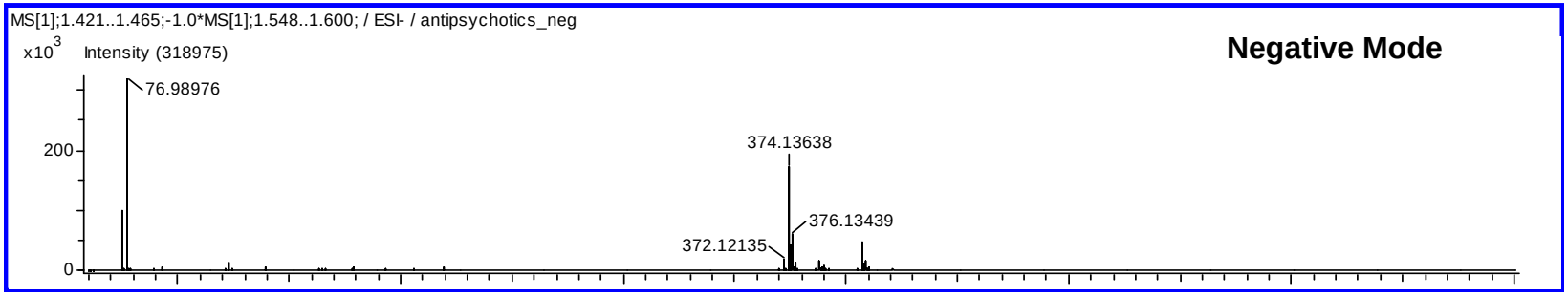




A-5. Antipsychotics (continued)

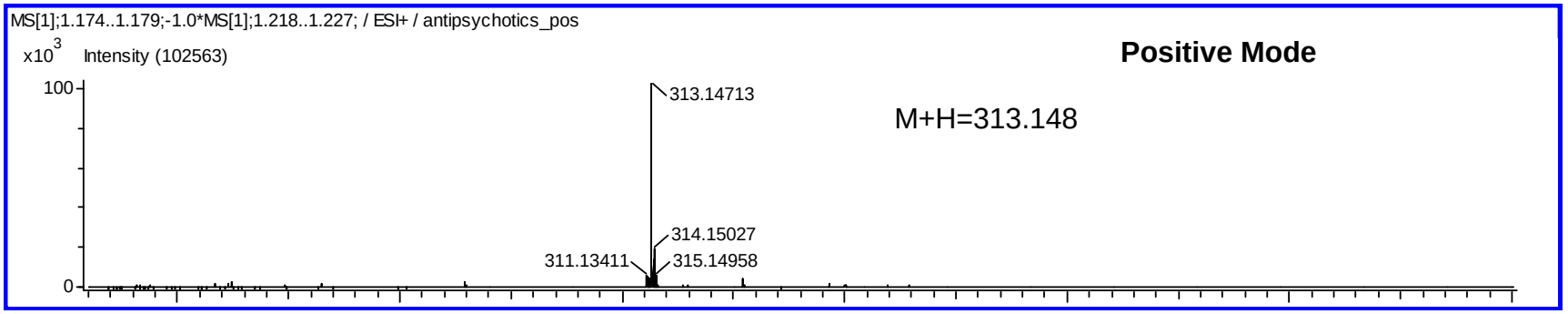
A-5.2. Haloperidol

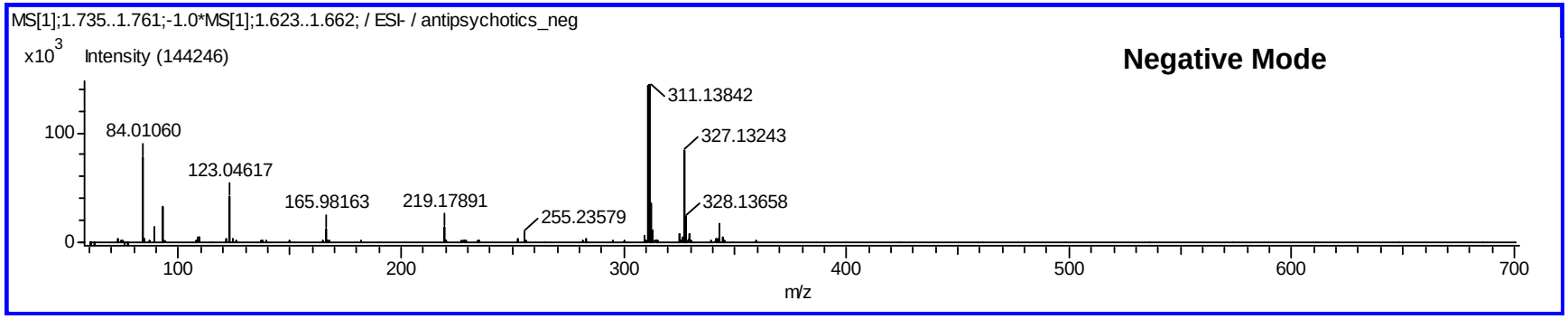




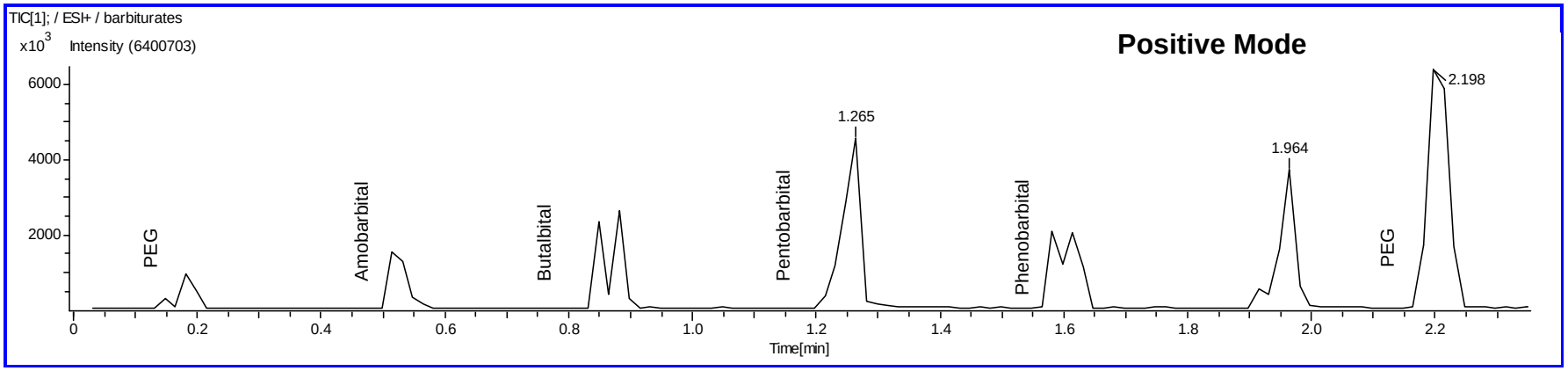
A-5. Antipsychotics (continued)

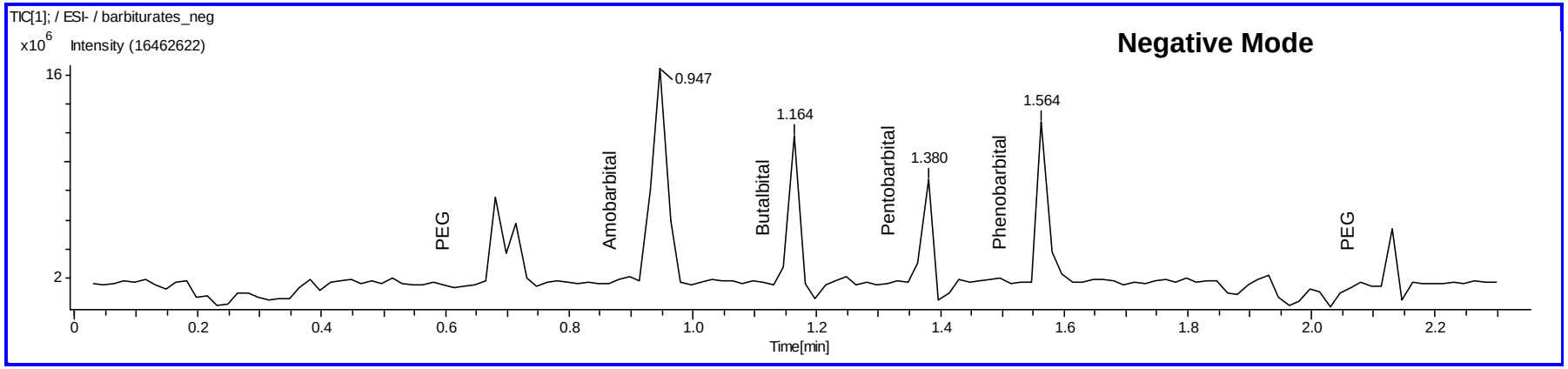
A-5.3. Olanzapine





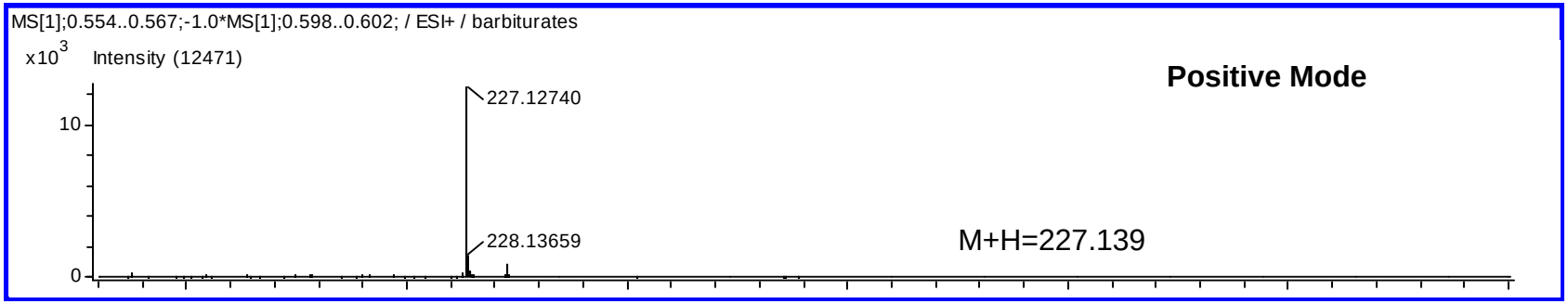
A-6. Barbiturates (4/18/07)

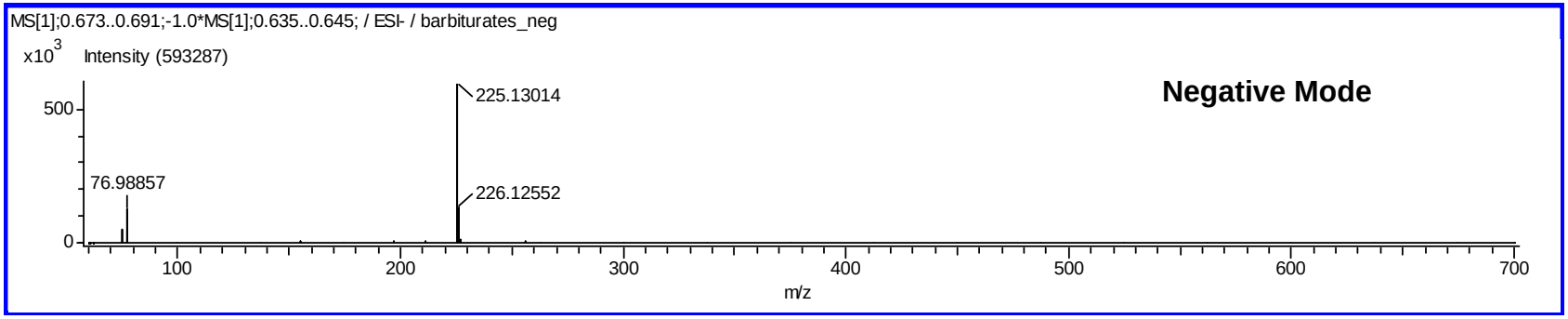




A-6. Barbiturates (continued)

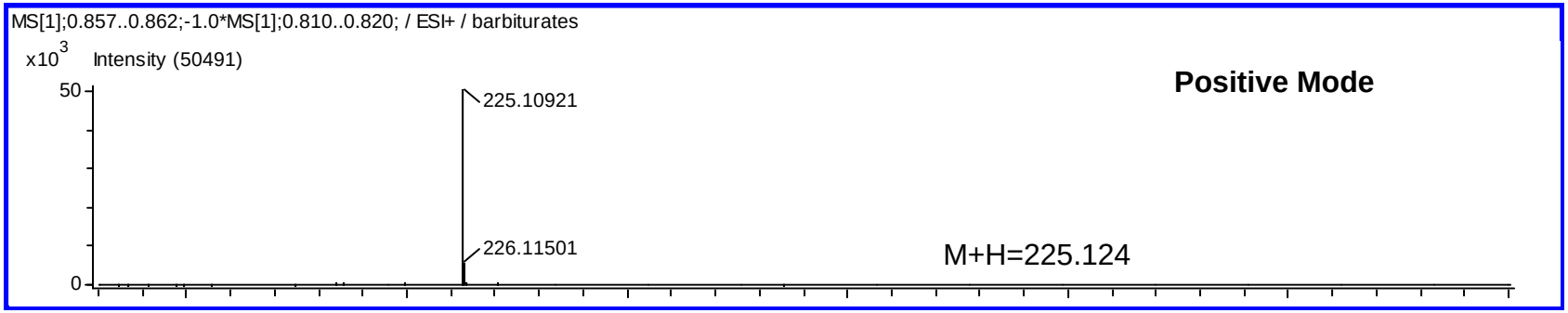
A-6.1. Amobarbital

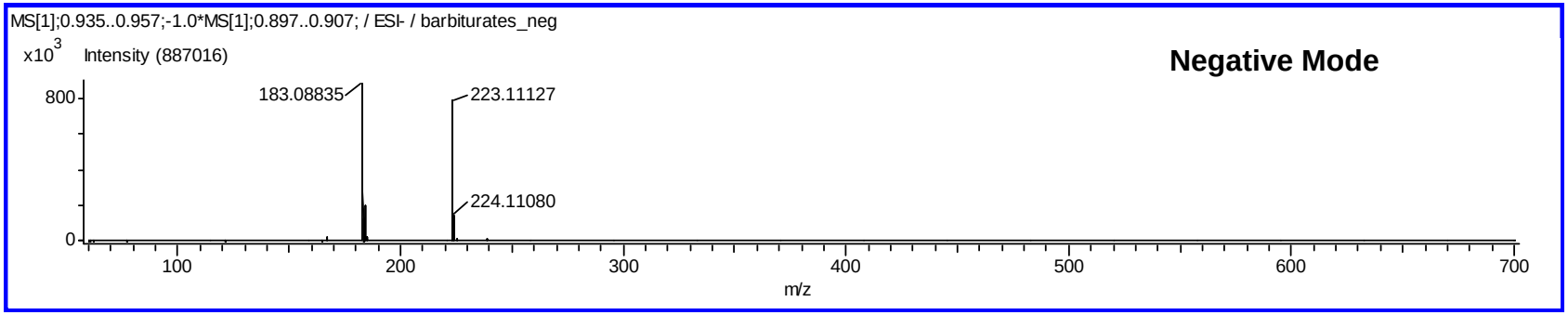




A-6. Barbiturates (continued)

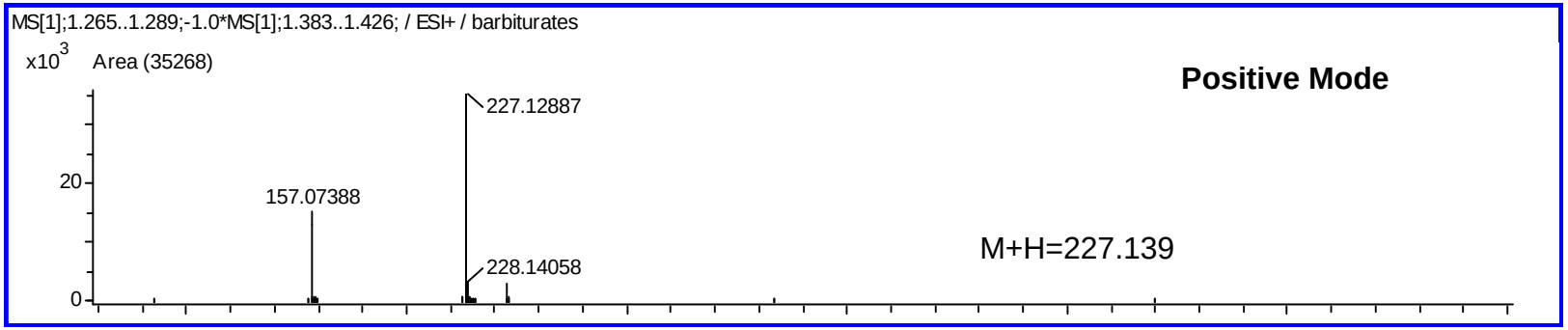
A-6.2. Butalbital

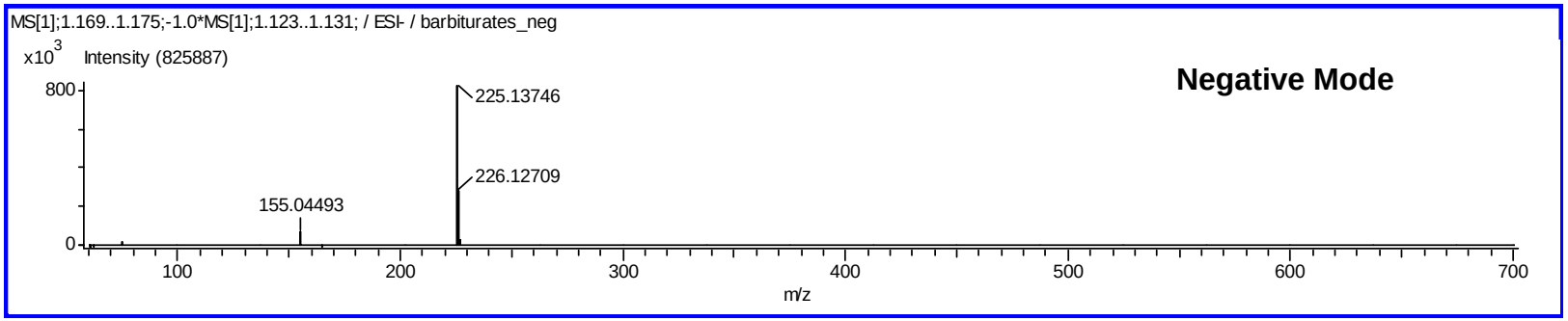




A-6. Barbiturates (continued)

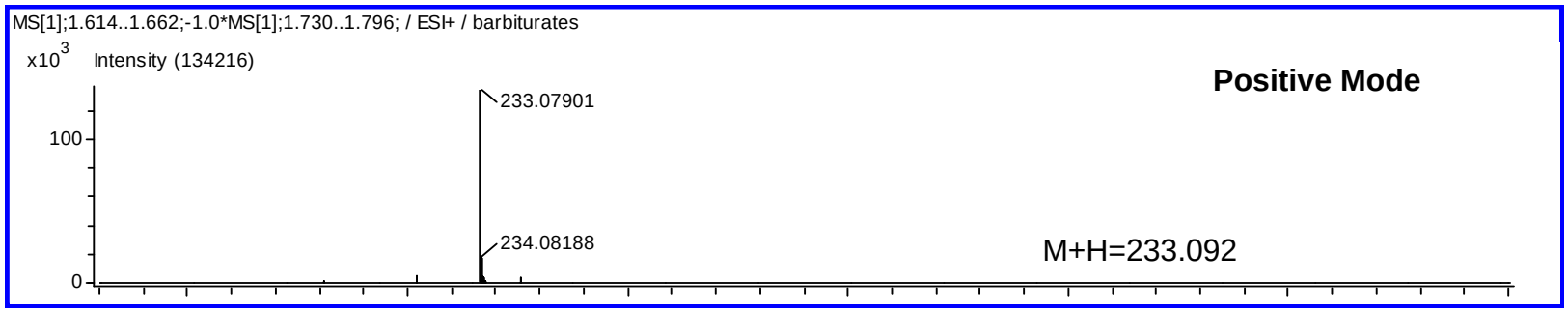
A-6.3. Pentobarbital

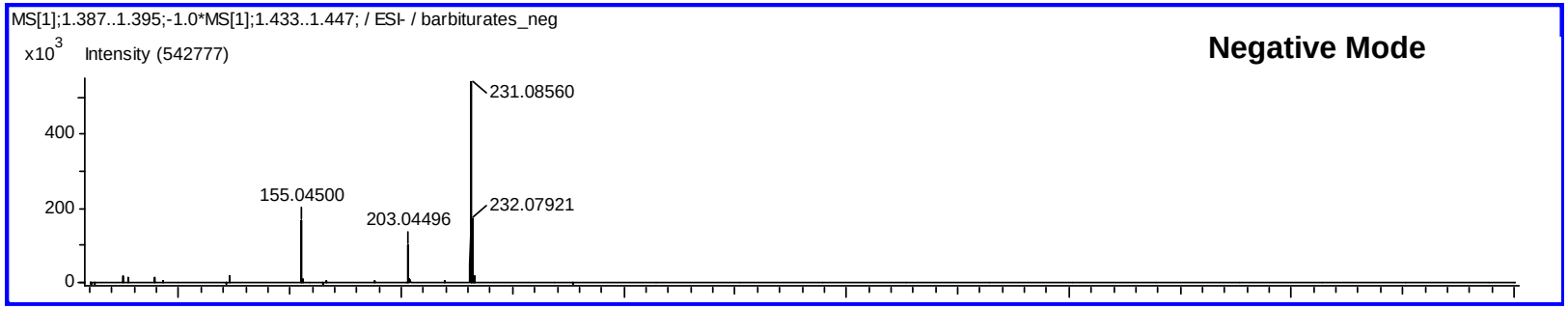




A-6. Barbiturates (continued)

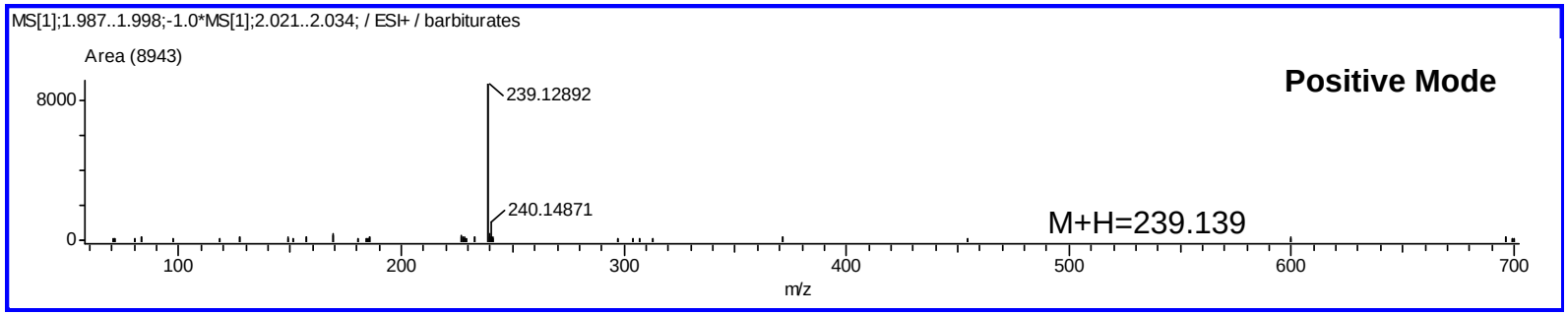
A-6.4. Phenobarbital

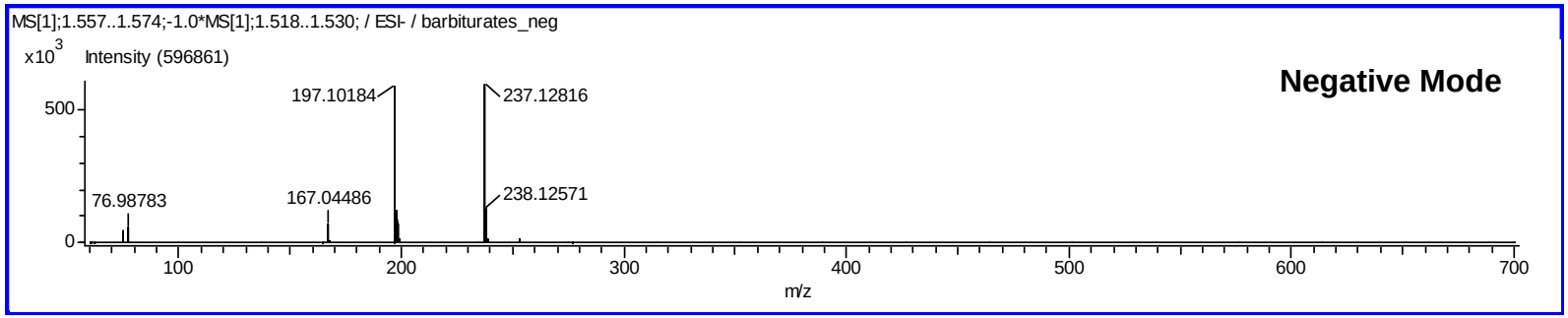




A-6. Barbiturates (continued)

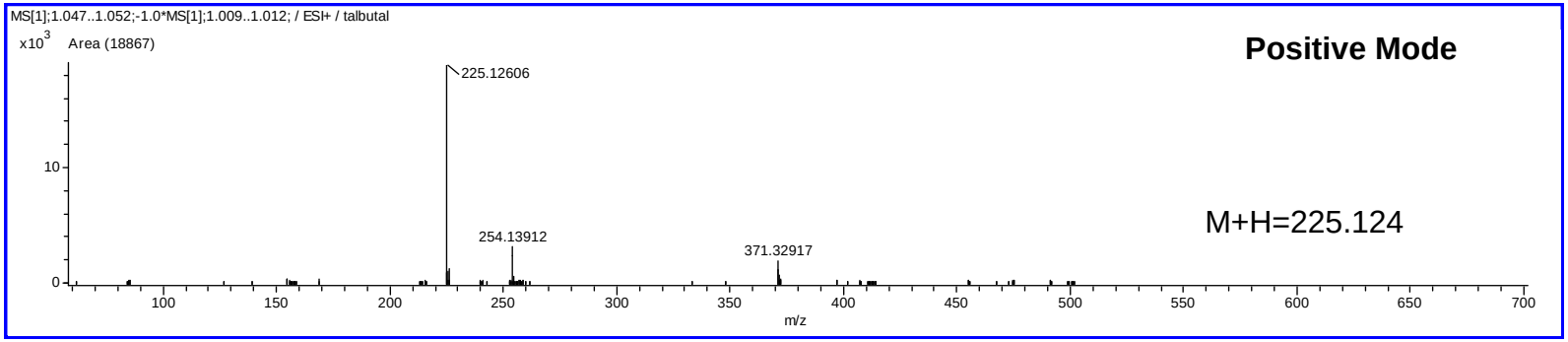
A-6.5. Secobarbital

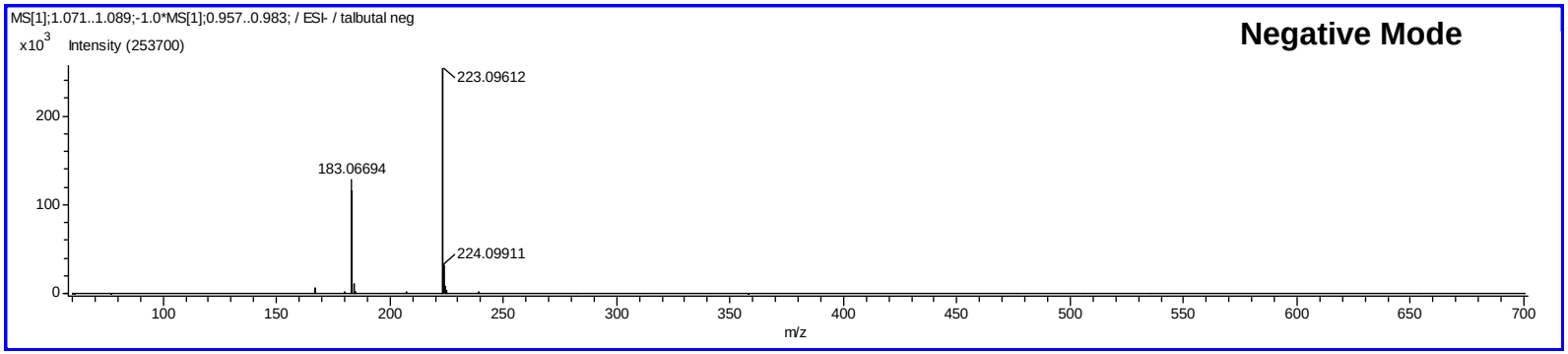




A-6. Barbiturates (continued)

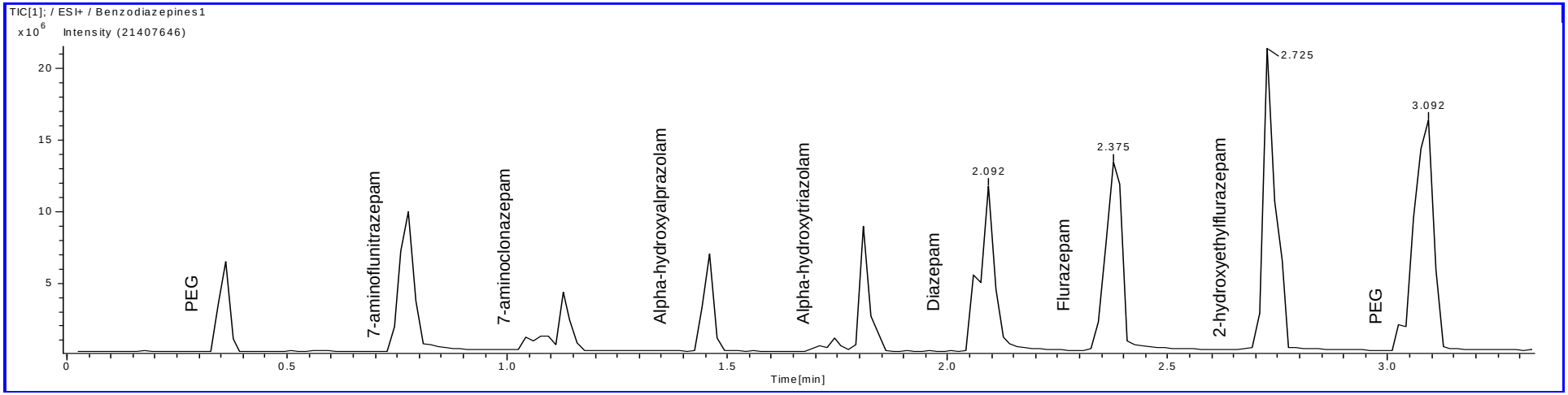
A-6.6. Talbutal

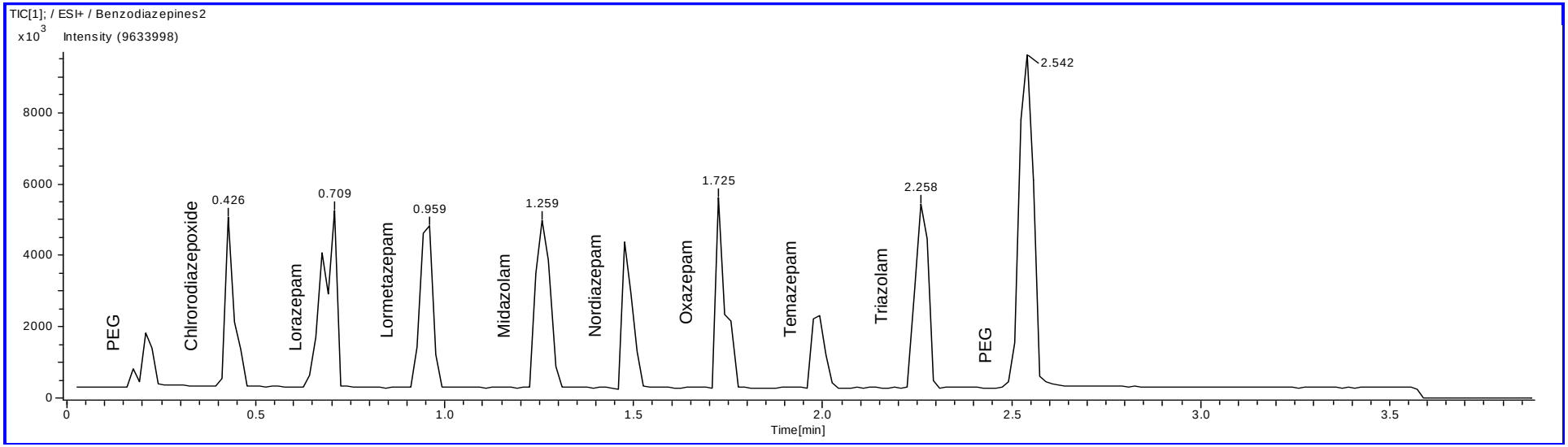




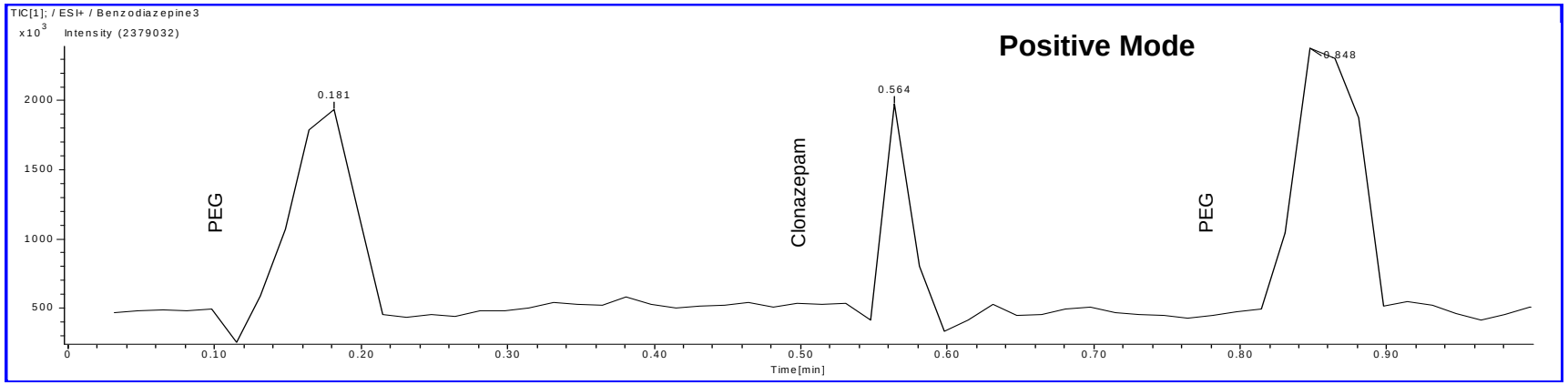
A-7. Benzodiazepines (4/11/07)

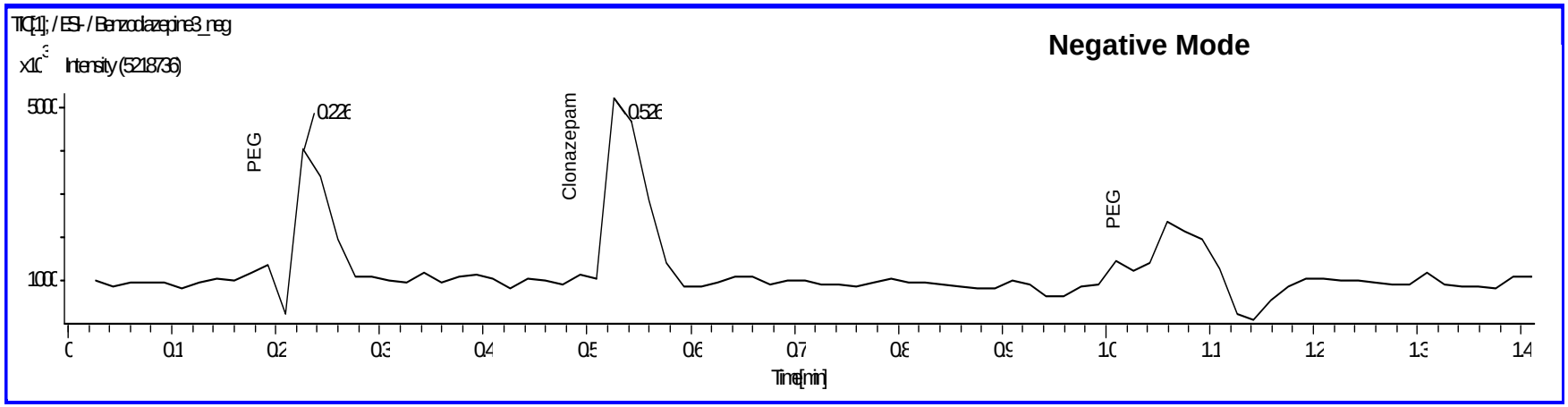
Positive Mode





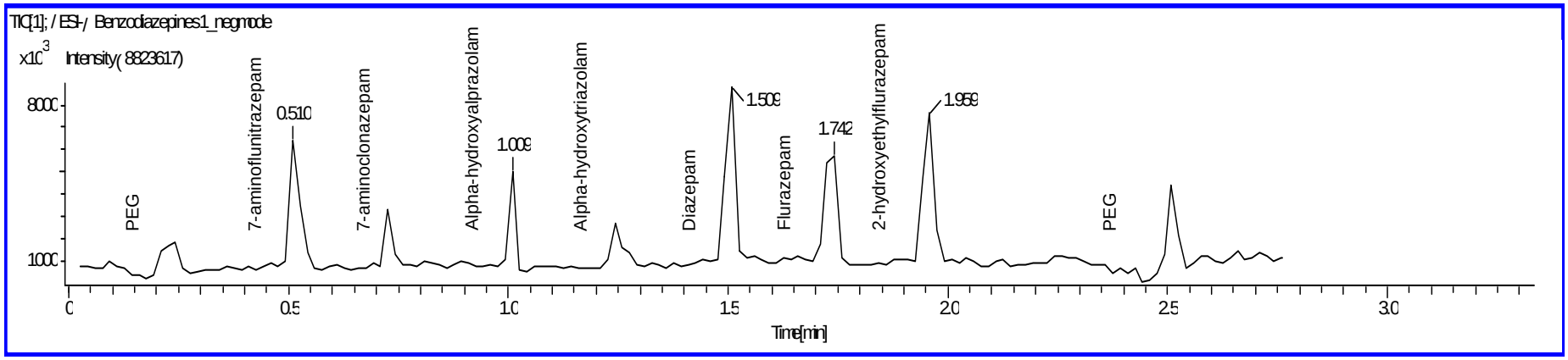
A-7. Benzodiazepines (continued) (4/26/07)

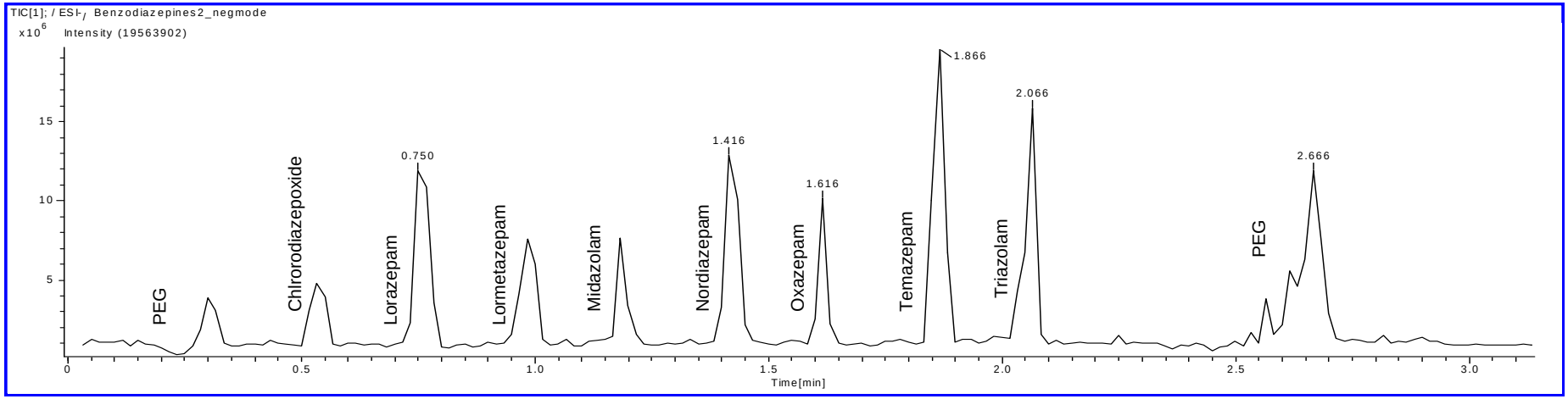




A-7. Benzodiazepines (continued) (4/11/07)

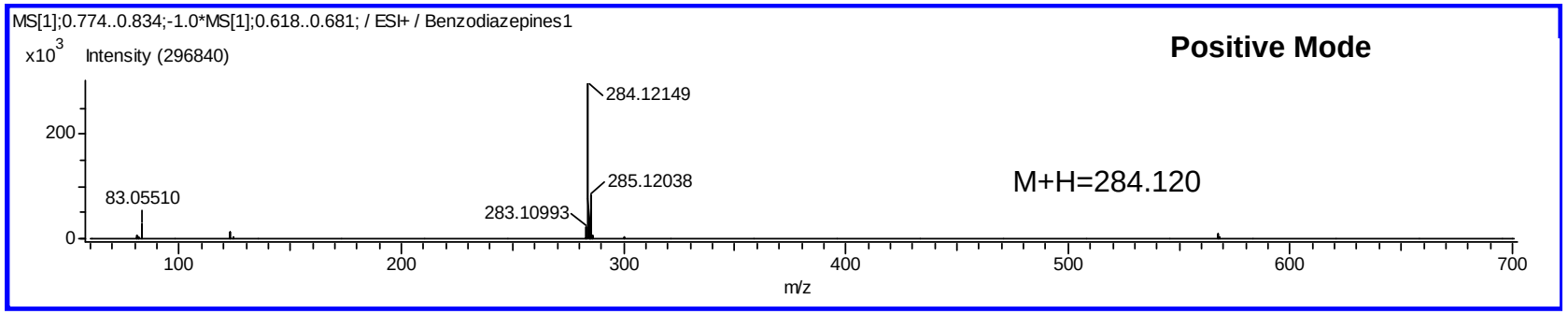
Negative Mode

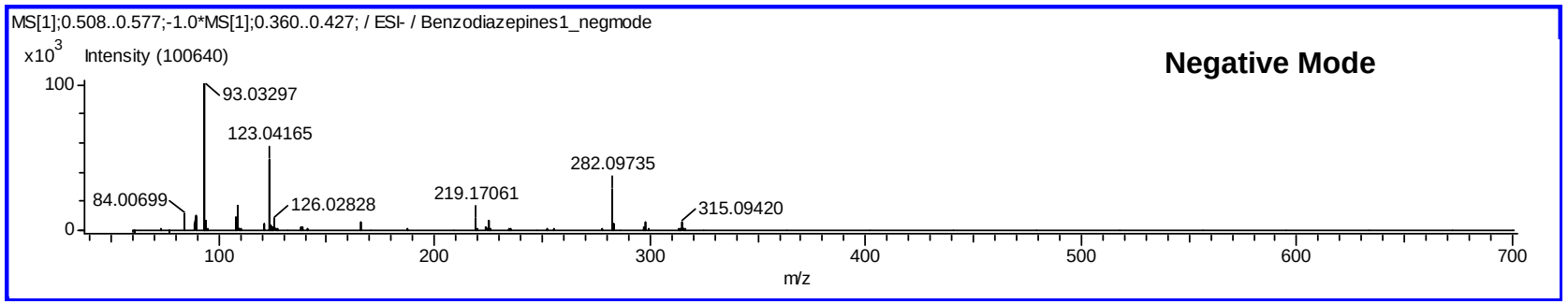




A-7. Benzodiazepines (continued)

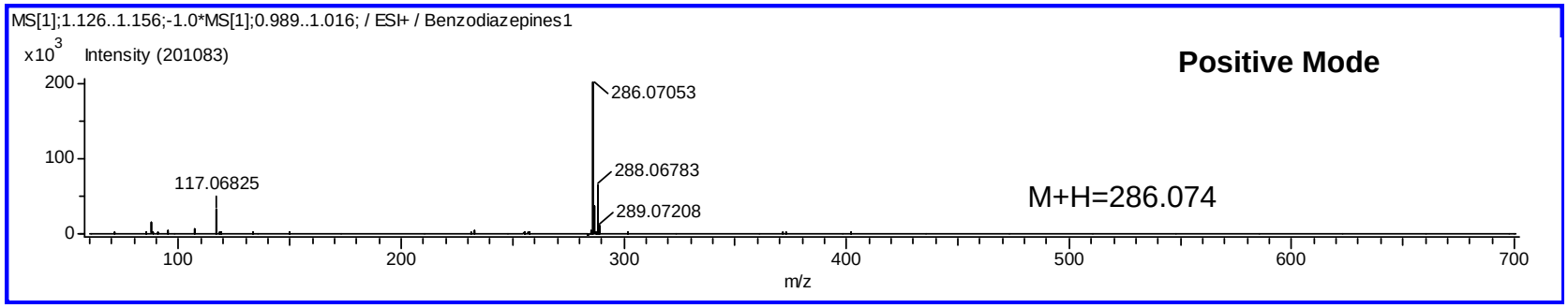
A-7.1. 7-aminoflunitrazepam

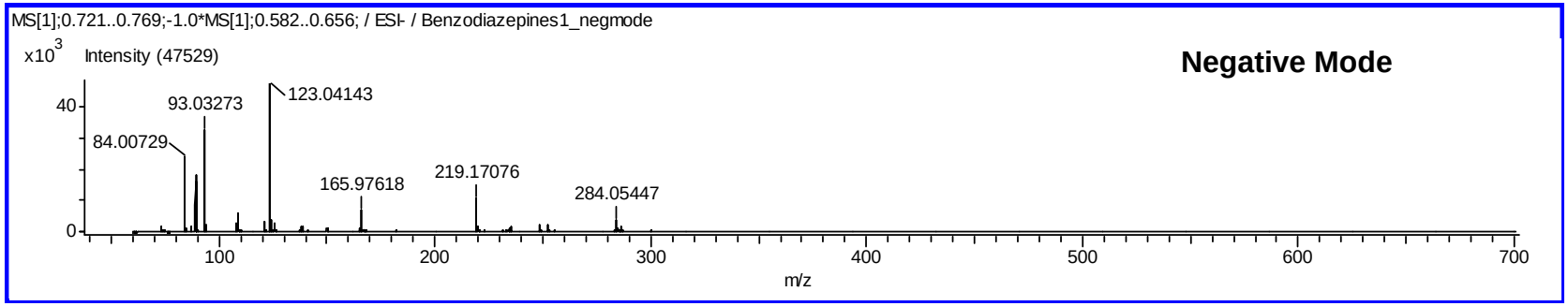




A-7. Benzodiazepines (continued)

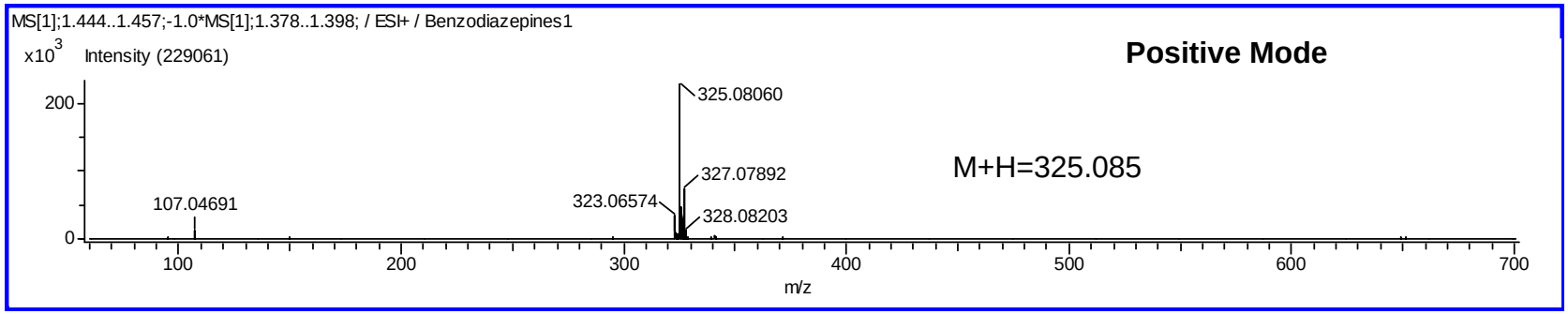
A-7.2. 7-aminoclonazepam

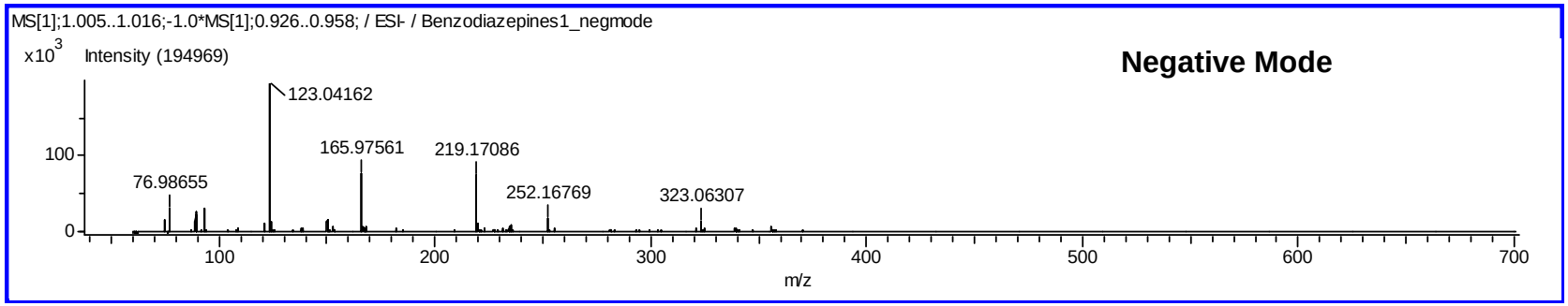




A-7. Benzodiazepines (continued)

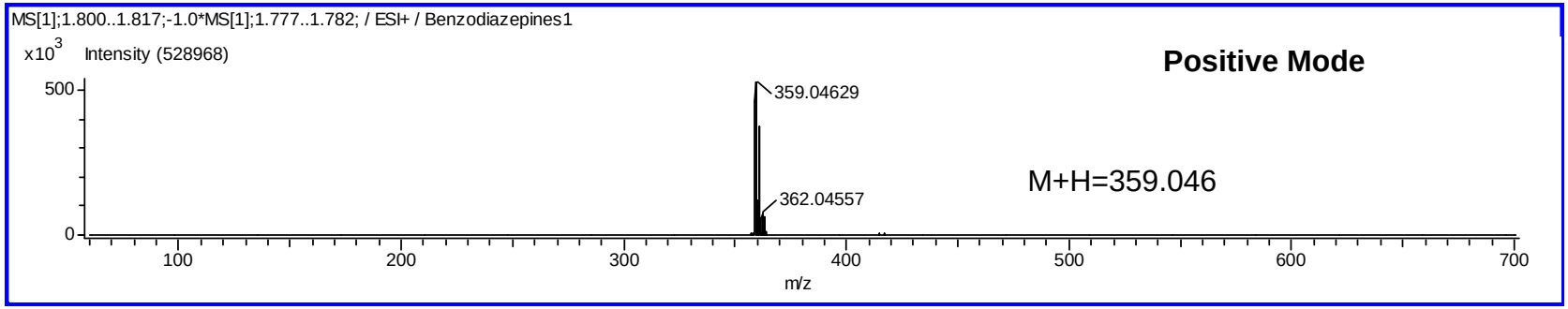
A-7.3. Alpha-hydroxyalprazolam

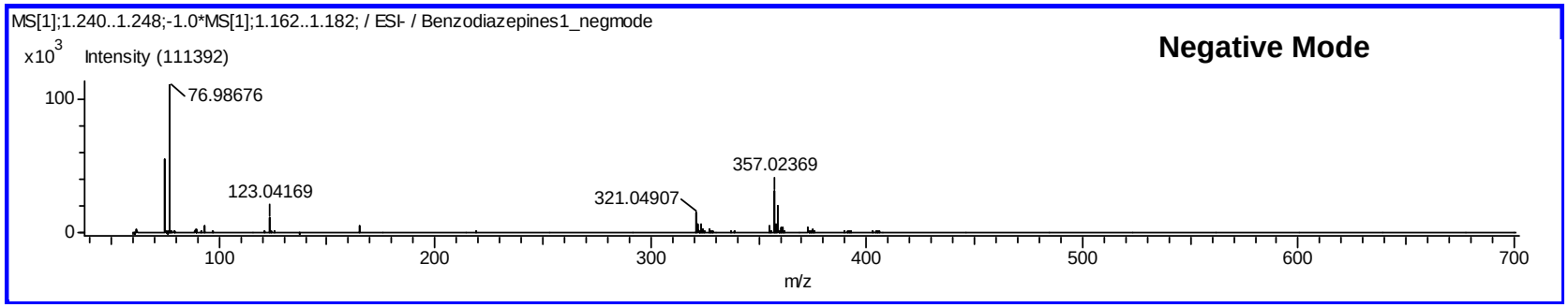




A-7. Benzodiazepines (continued)

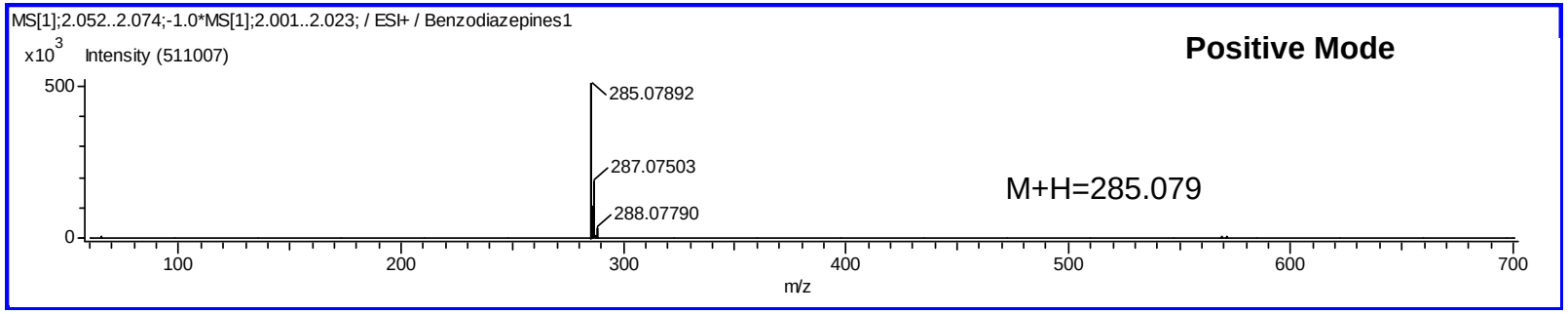
A-7.4. Alpha-hydroxytriazolam

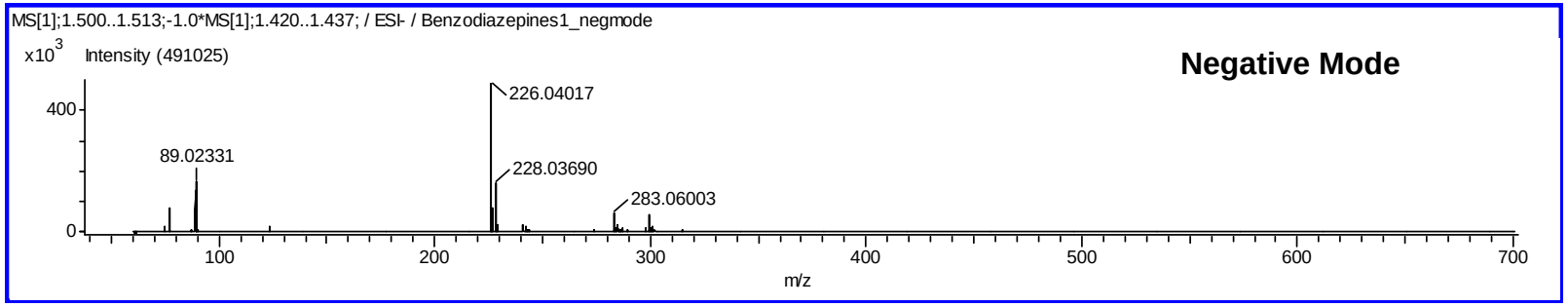




A-7. Benzodiazepines (continued)

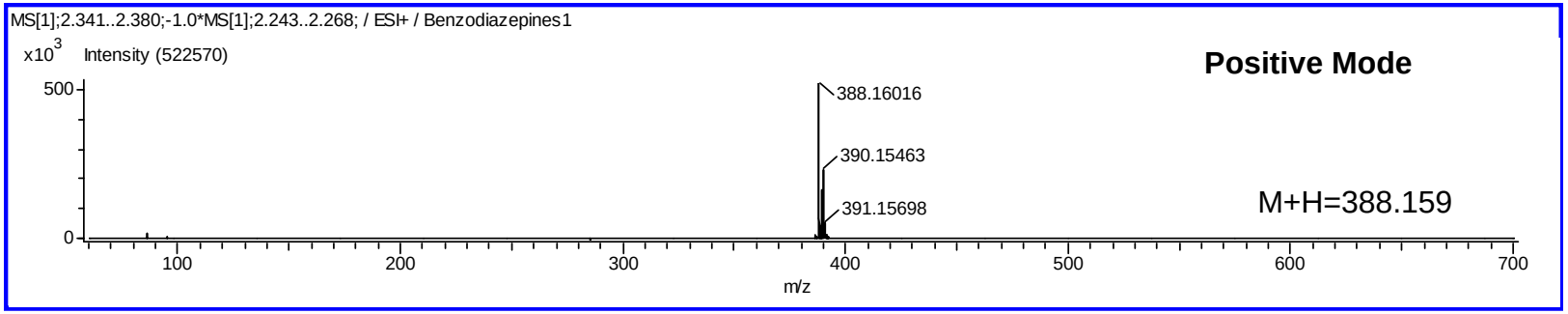
A-7.5. Diazepam

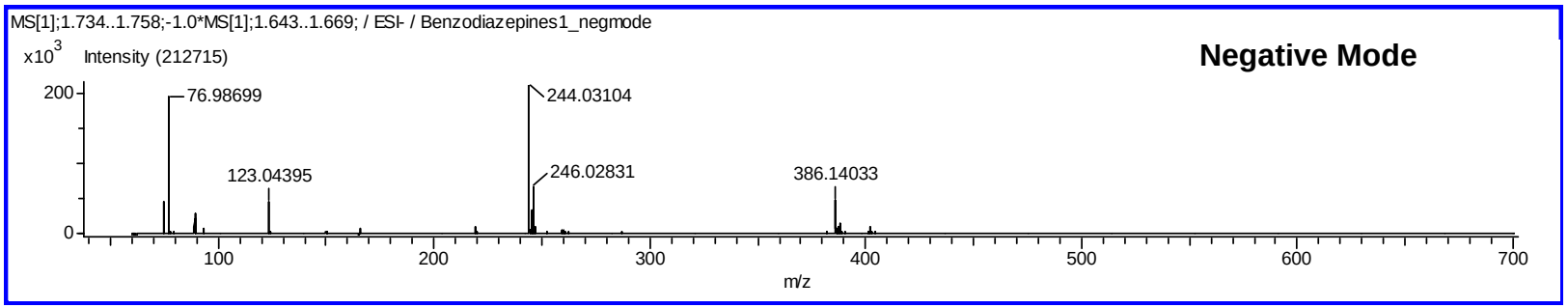




A-7. Benzodiazepines (continued)

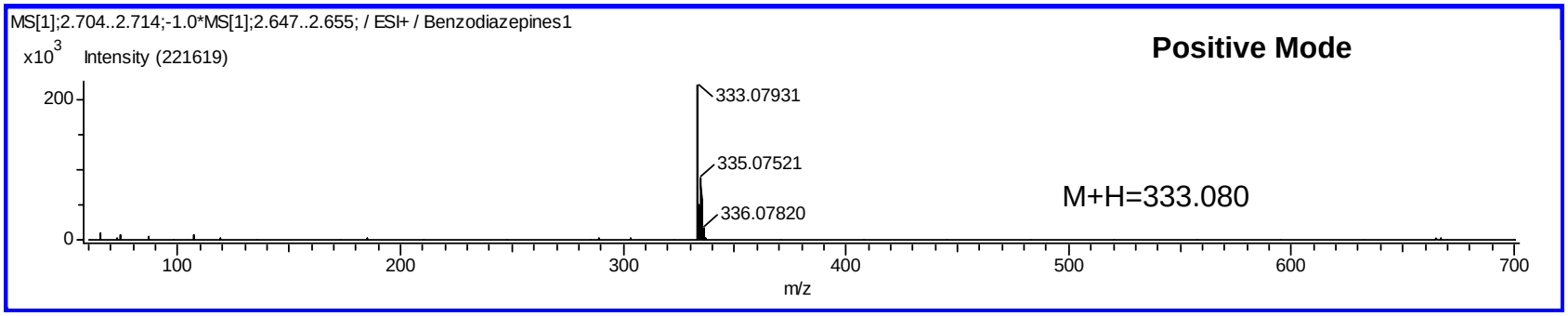
A-7.6. Flurazepam

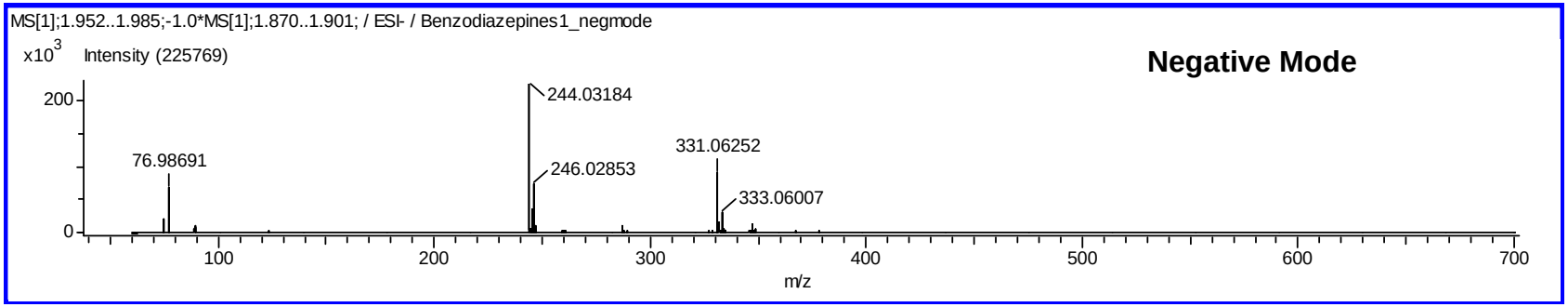




A-7. Benzodiazepines (continued)

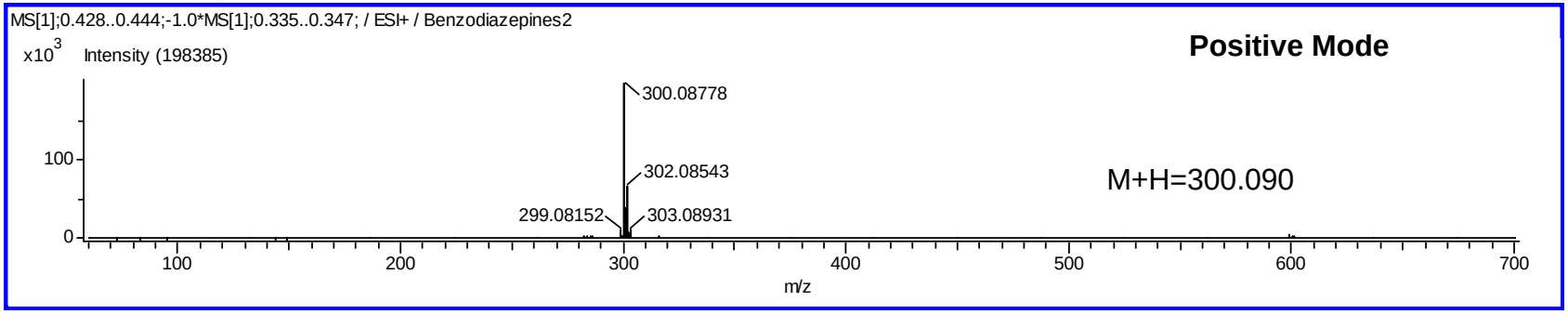
A-7.7. 2-hydroxyethylflurazepam

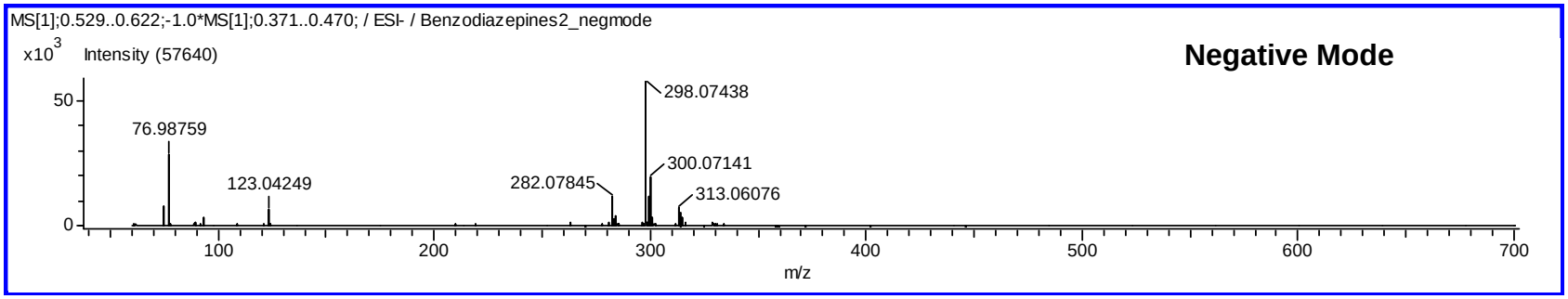




A-7. Benzodiazepines (continued)

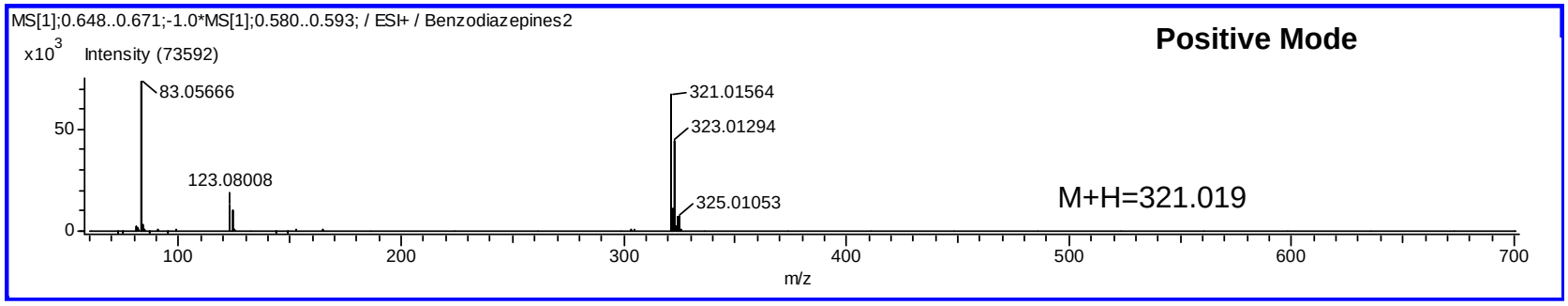
A-7.8. Chlorodiazepoxide

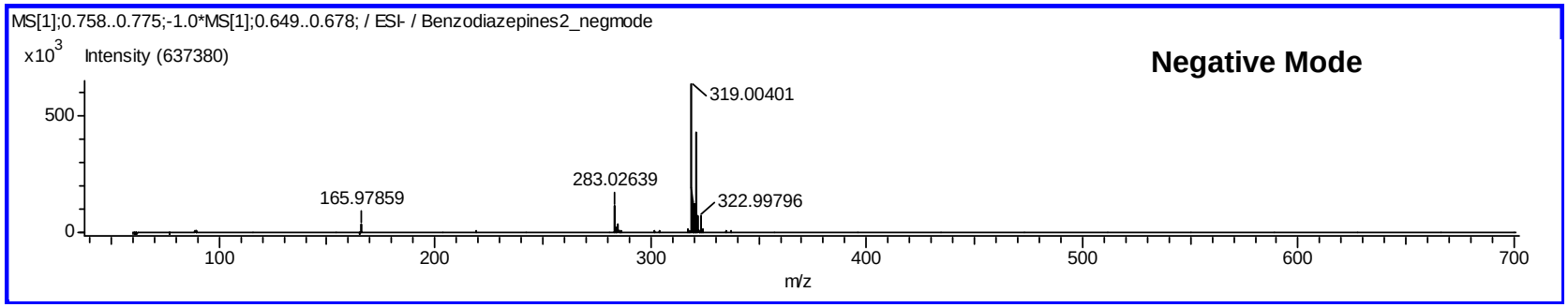




A-7. Benzodiazepines (continued)

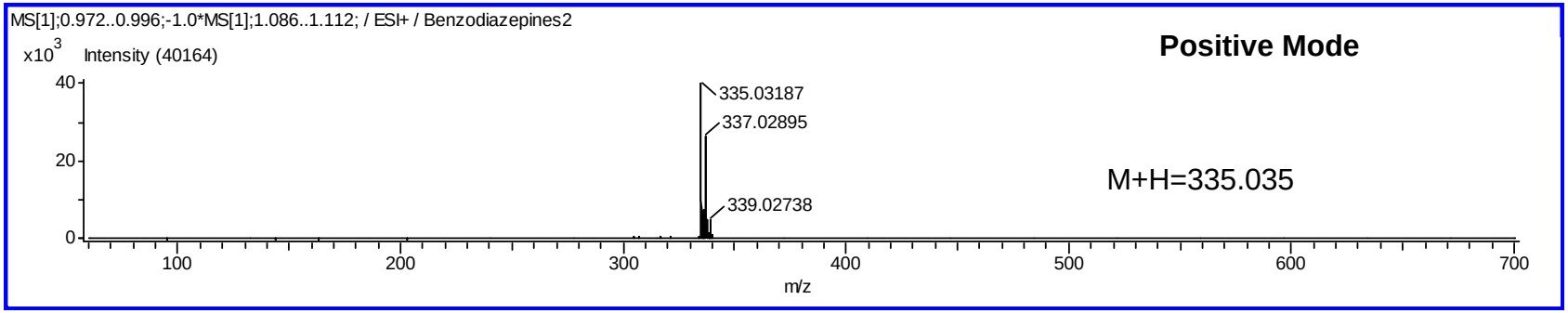
A-7.9. Lorazepam

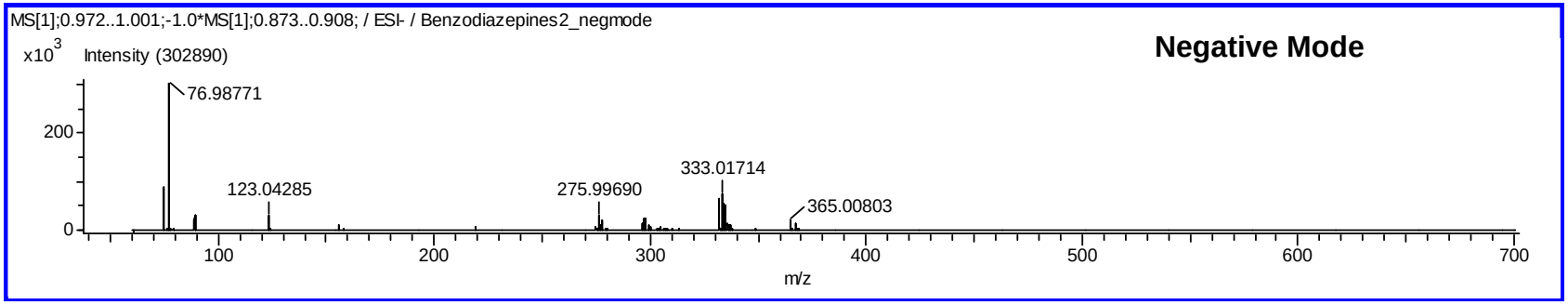




A-7. Benzodiazepines (continued)

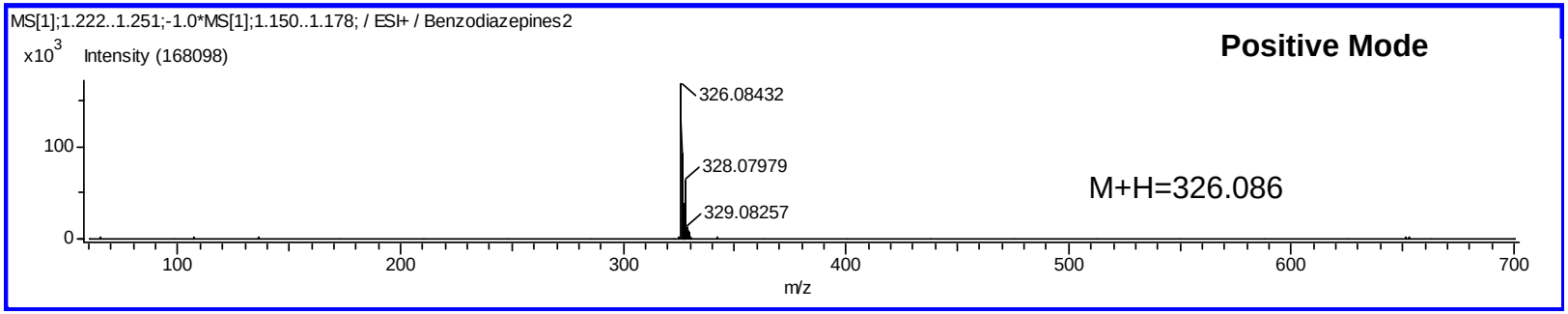
A-7.10. Lormetazepam

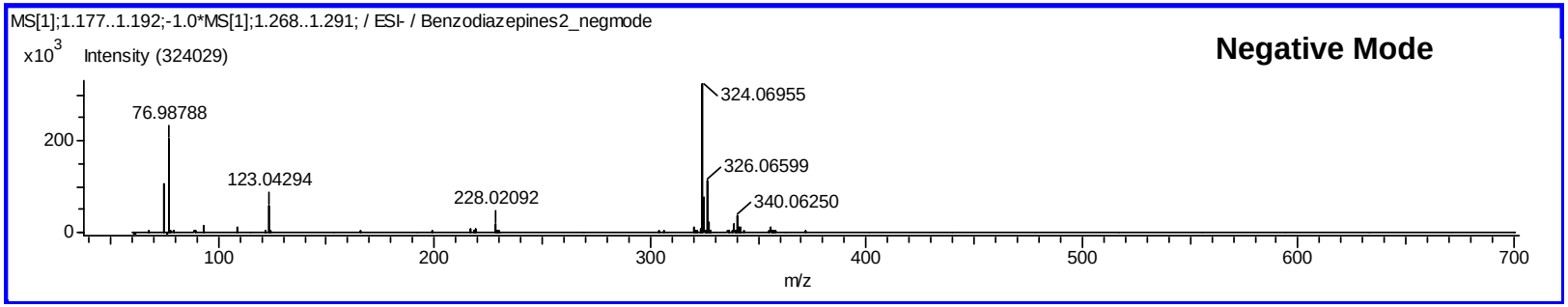




A-7. Benzodiazepines (continued)

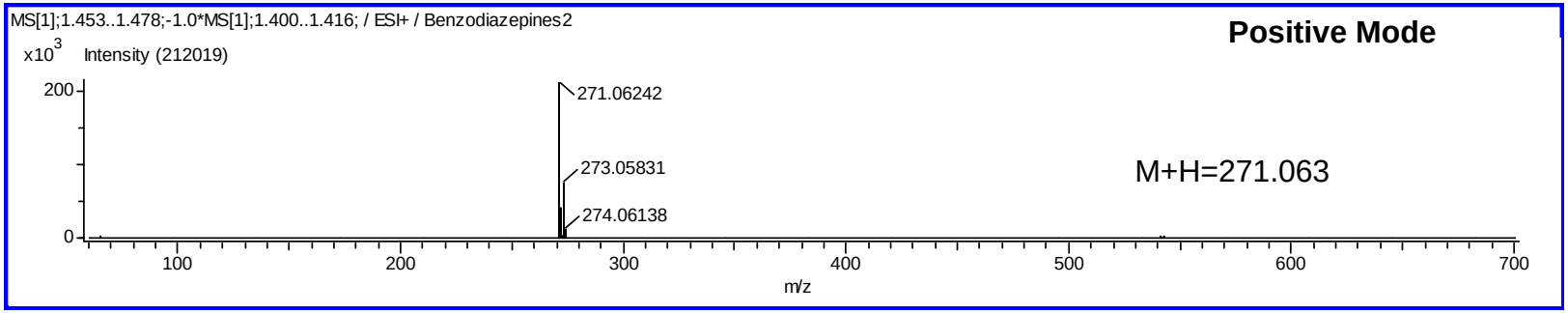
A-7.11. Midazolam

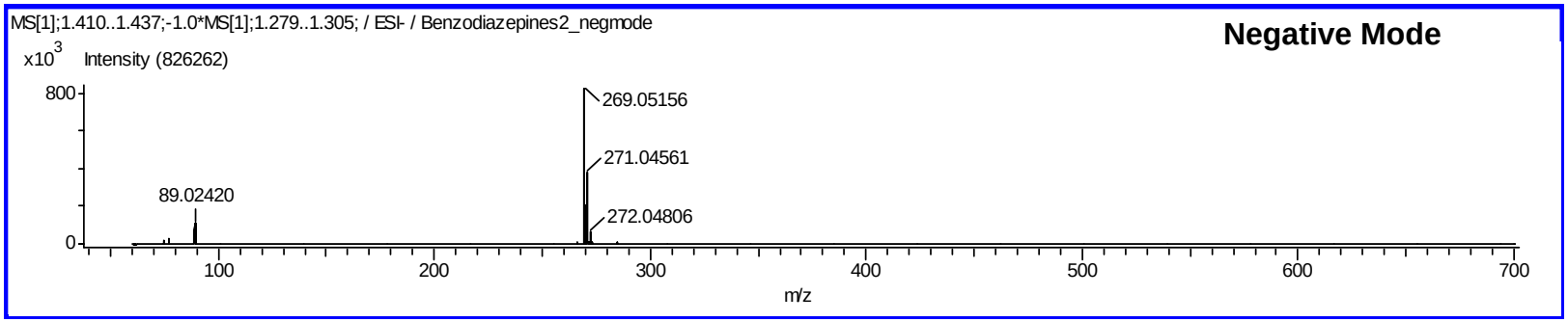




A-7. Benzodiazepines (continued)

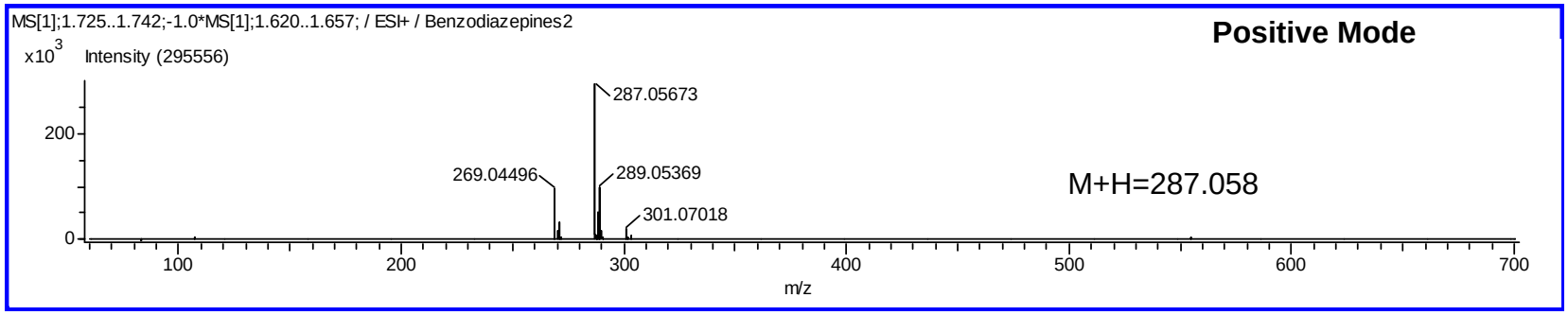
A-7.12. Nordiazepam

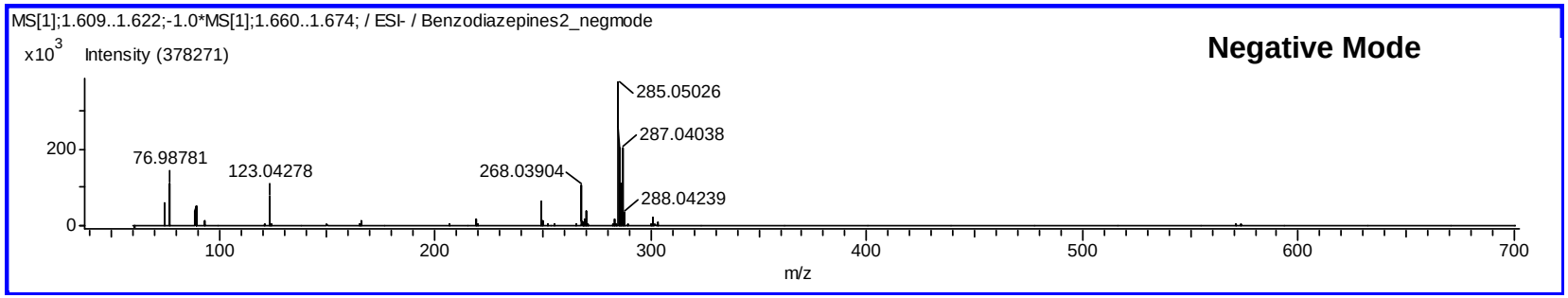




A-7. Benzodiazepines (continued)

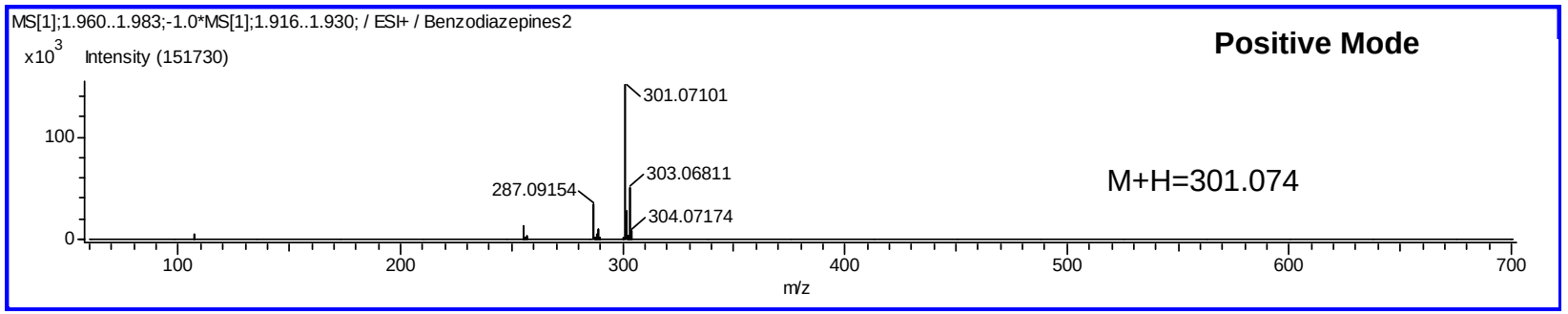
A-7.13. Oxazepam

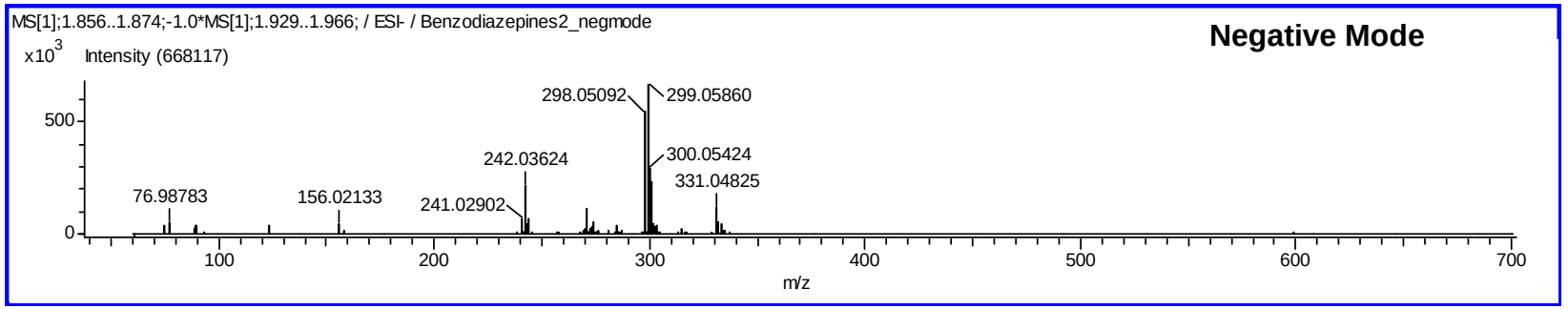




A-7. Benzodiazepines (continued)

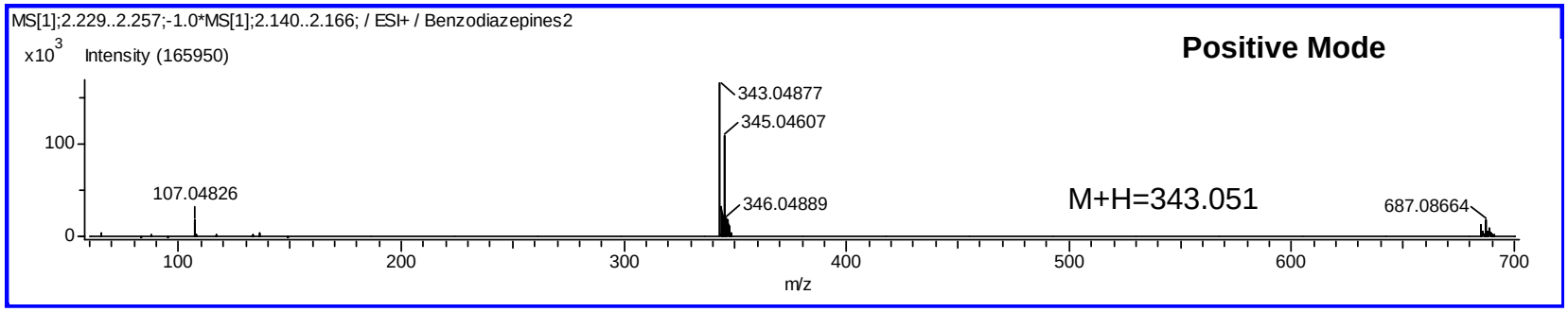
A-7.14. Temazepam

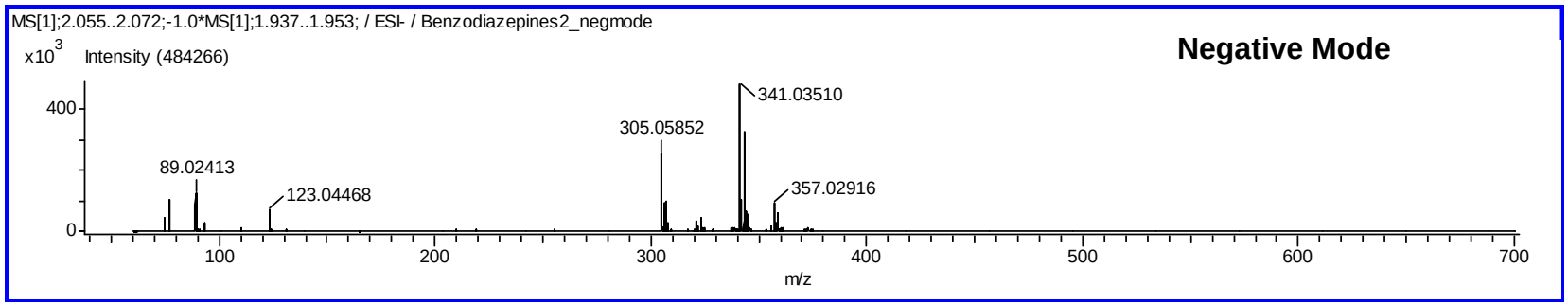




A-7. Benzodiazepines (continued)

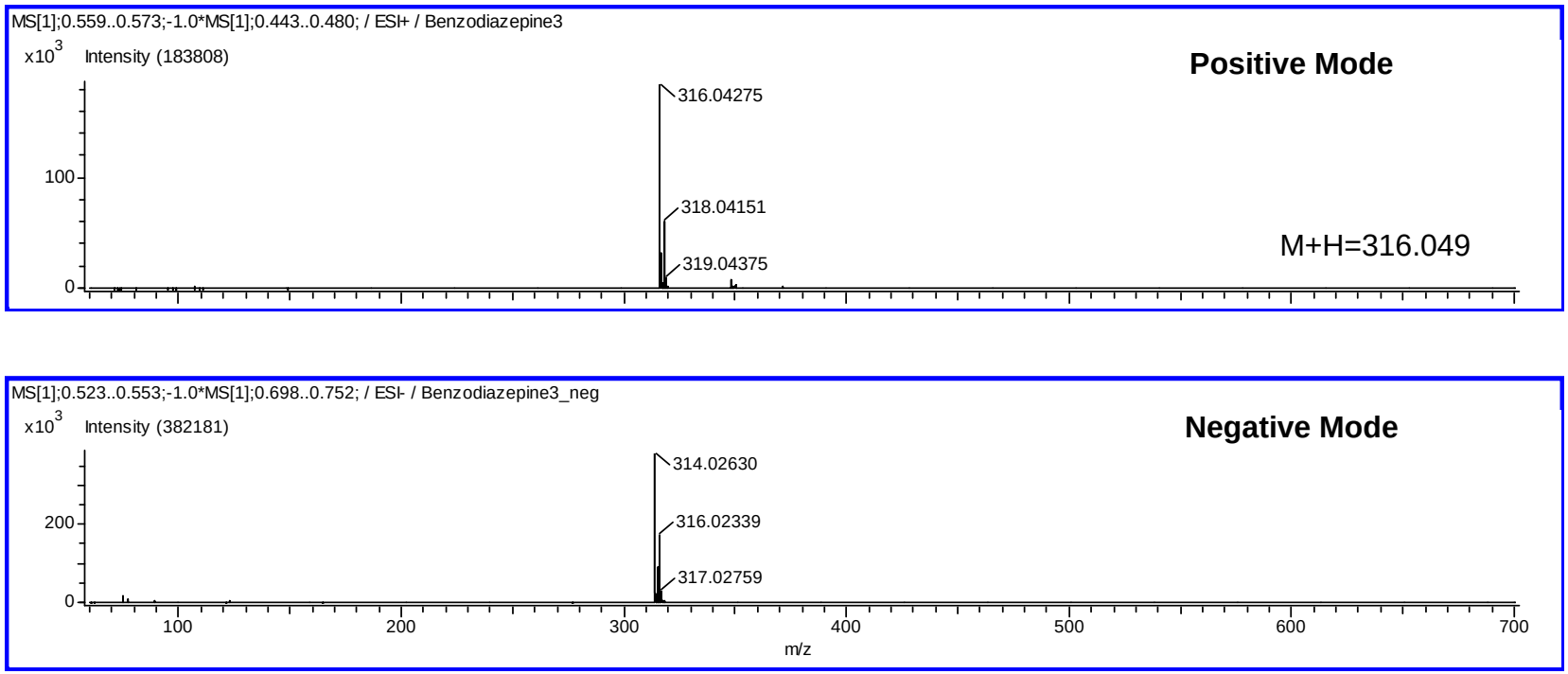
A-7.15. Triazolam





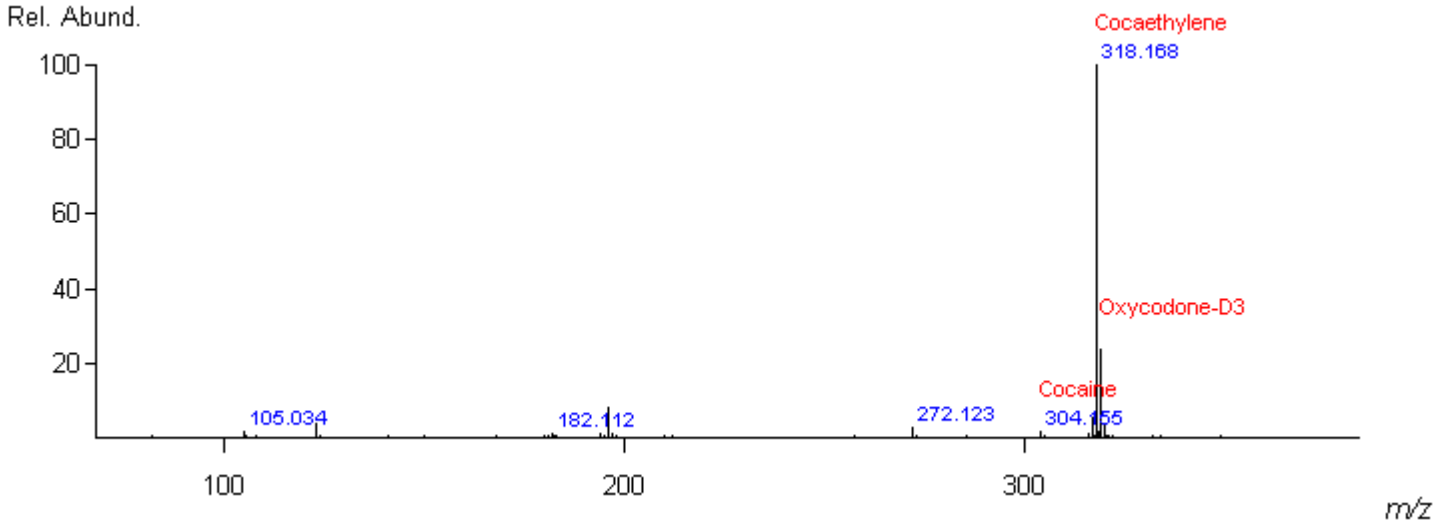
A-7. Benzodiazepines (continued)

A-7.16. Clonazepam



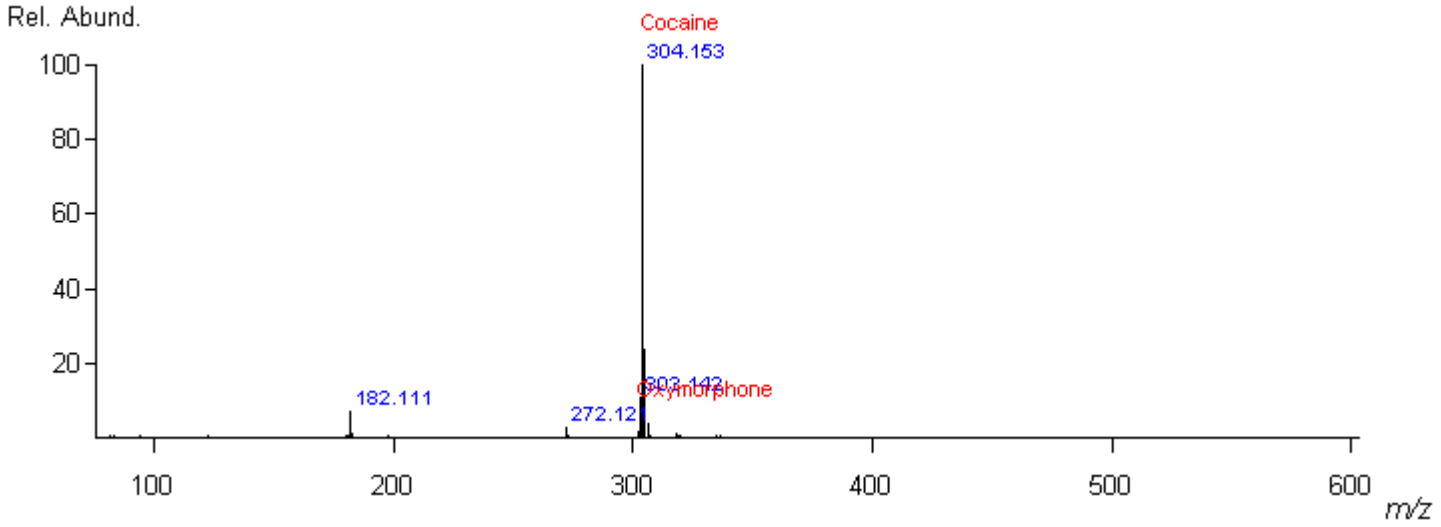
A-8. Cocaine Metabolites

A-8.1. Cocaethylene



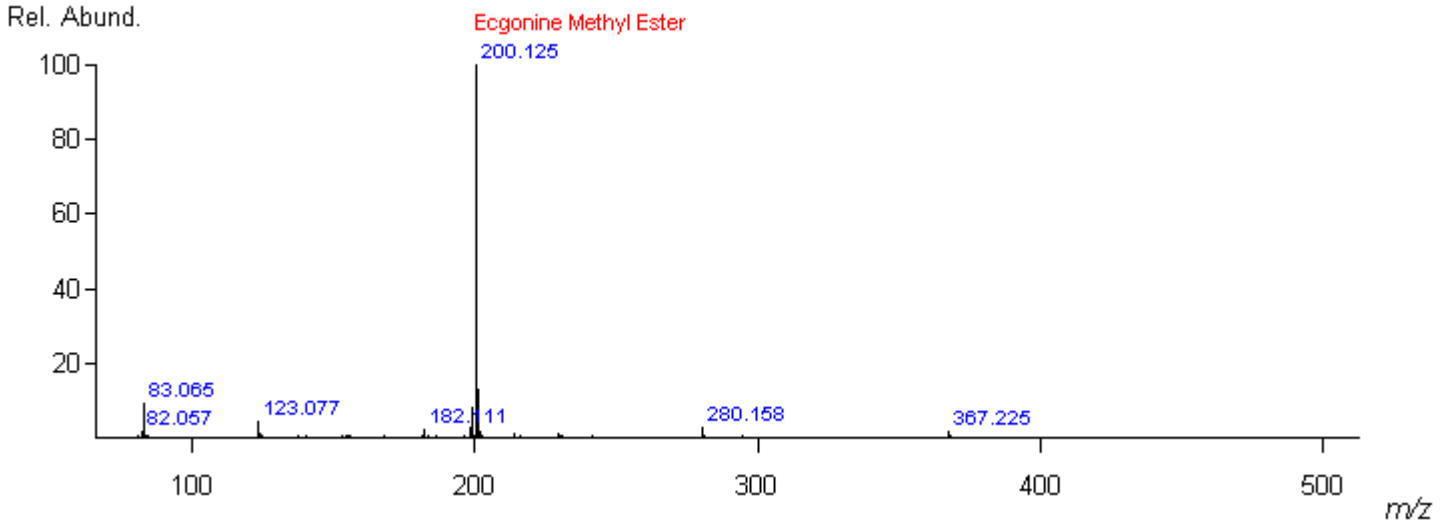
A-8. Cocaine Metabolites (continued)

A-8.2. Cocaine



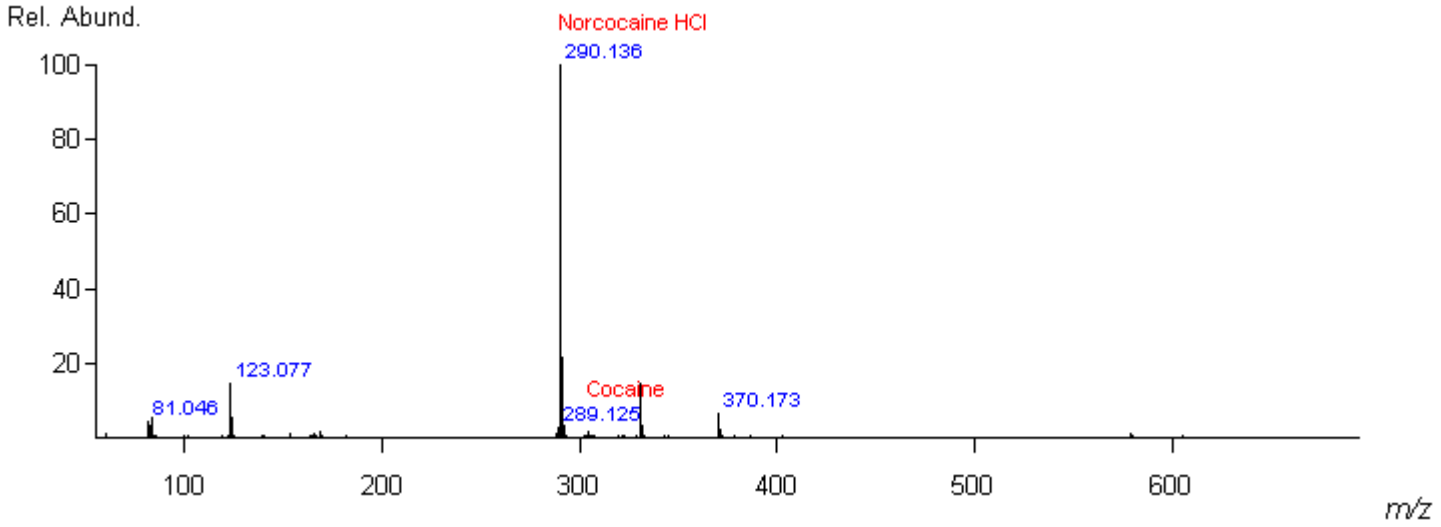
A-8. Cocaine Metabolites (continued)

A-8.3. Ecgonine Methyl Ester



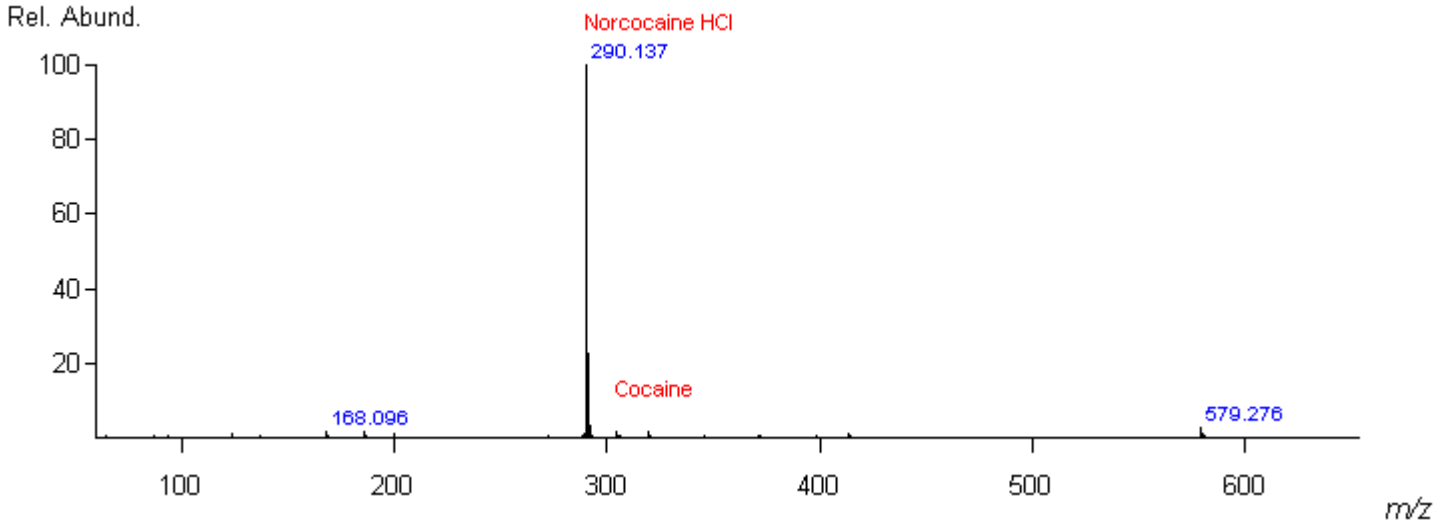
A-8. Cocaine Metabolites (continued)

A-8.4. Norcocaine



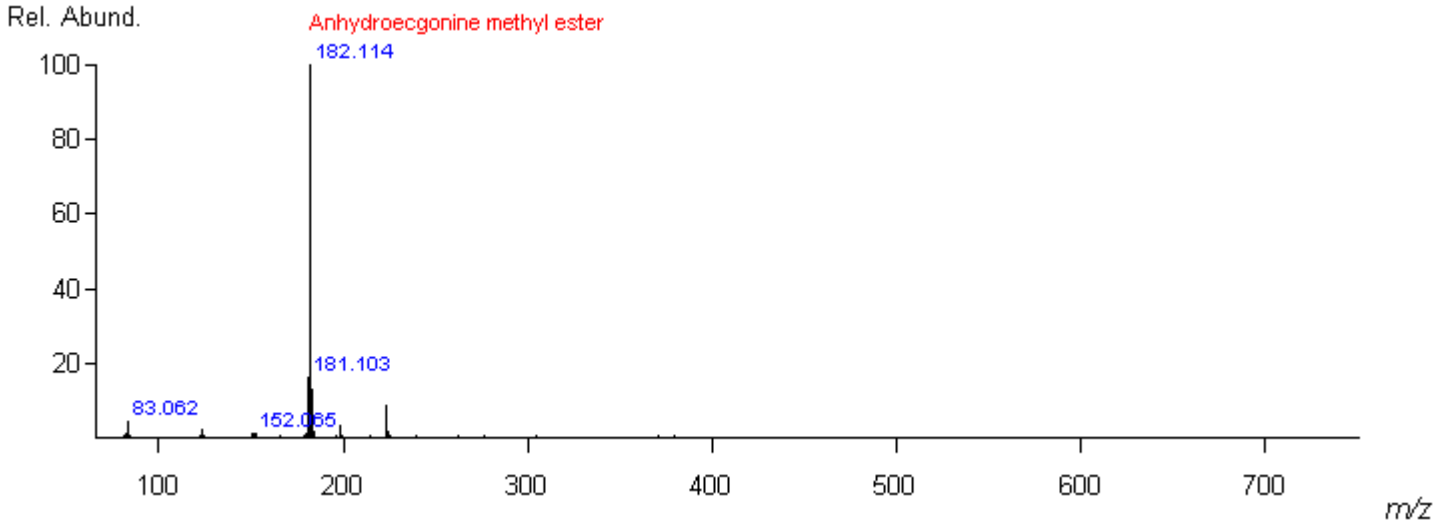
A-8. Cocaine Metabolites (continued)

A-8.5. Benzoylecgonine

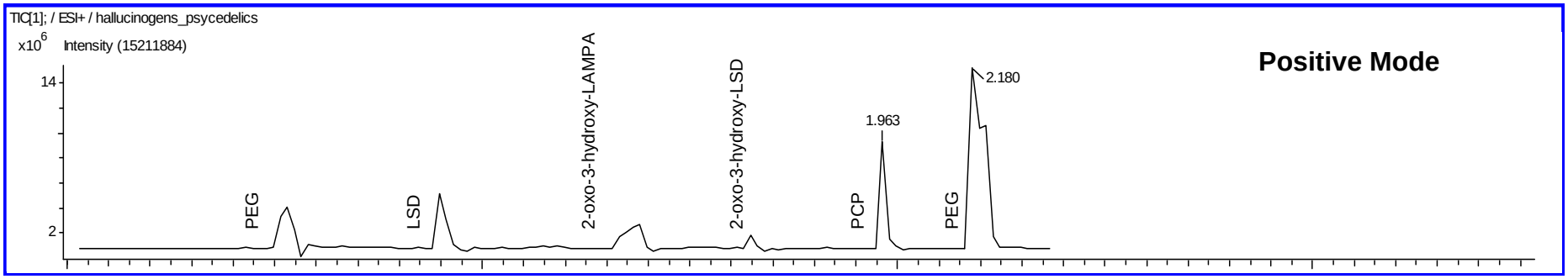


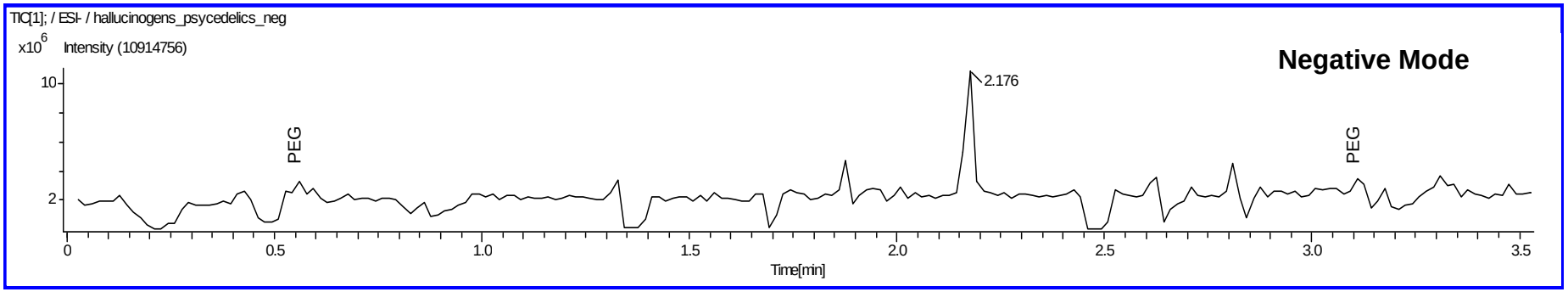
A-8. Cocaine Metabolites (continued)

A-8.6. Anhydroecgonine Methyl Ester

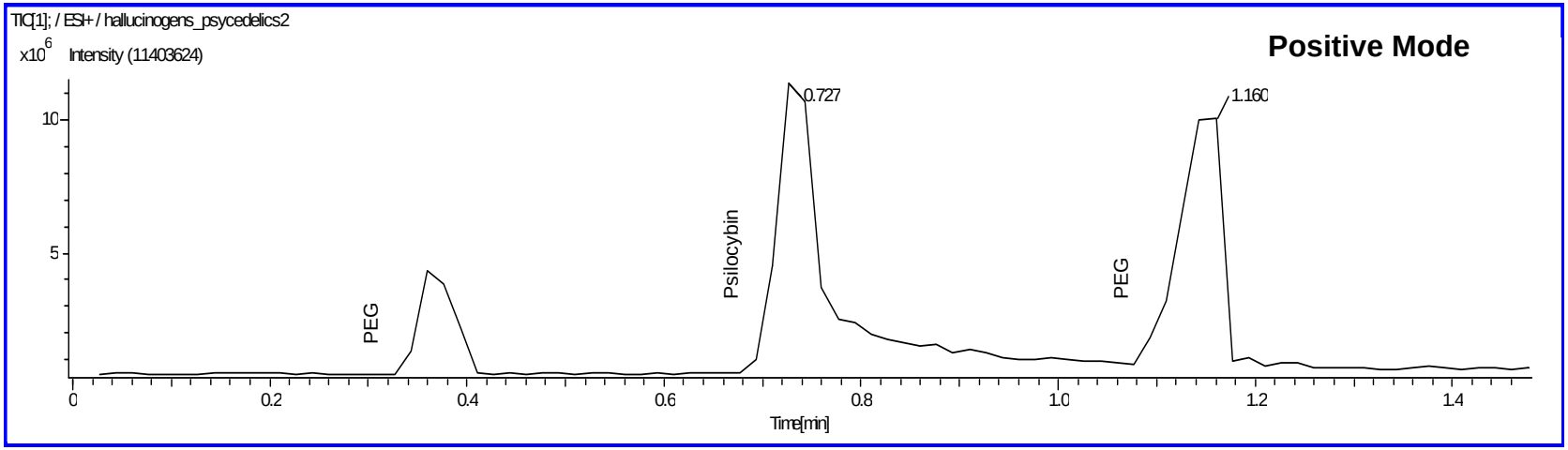


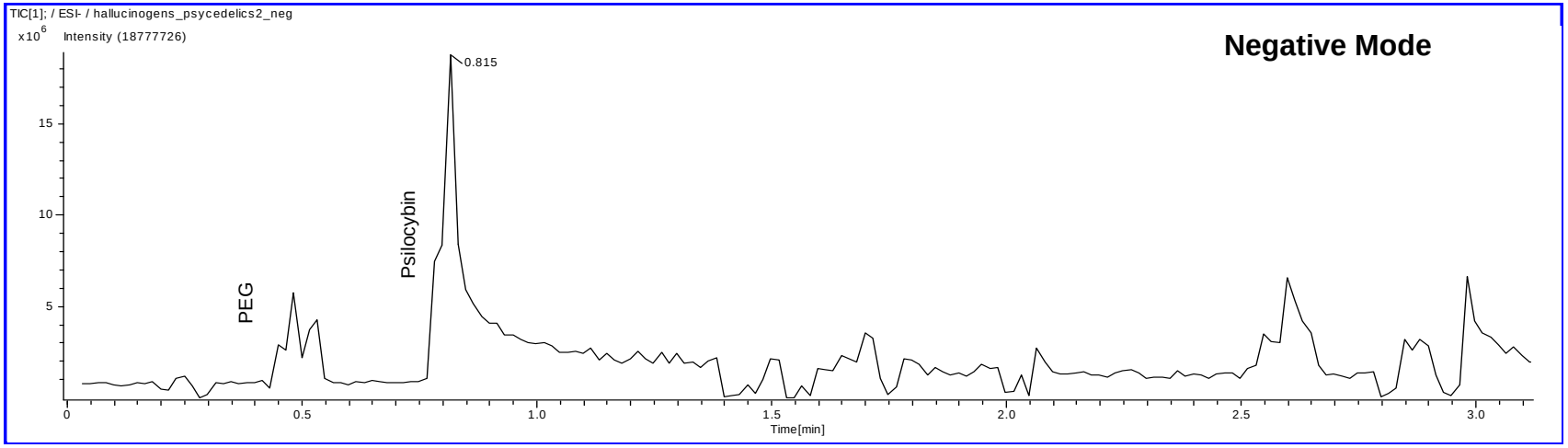
A-9. Hallucinogens/Psychedelics (4/18/07)





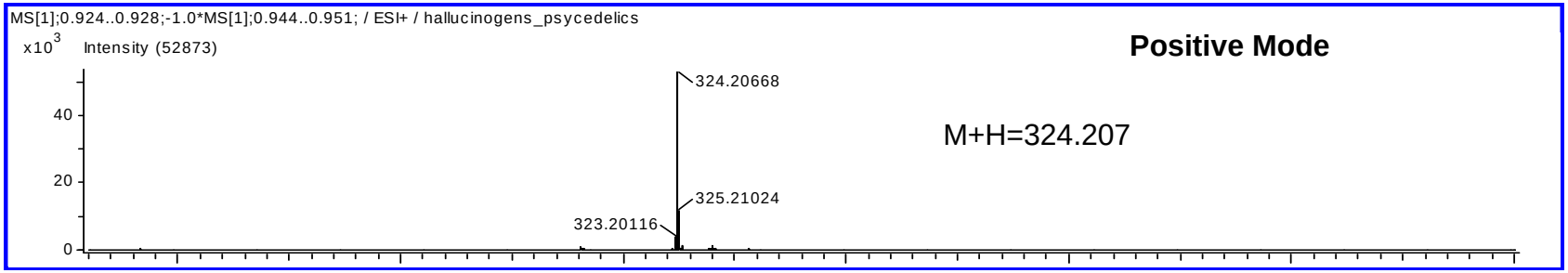
A-9. Hallucinogens/Psychedelics (continued) (4/26/07)

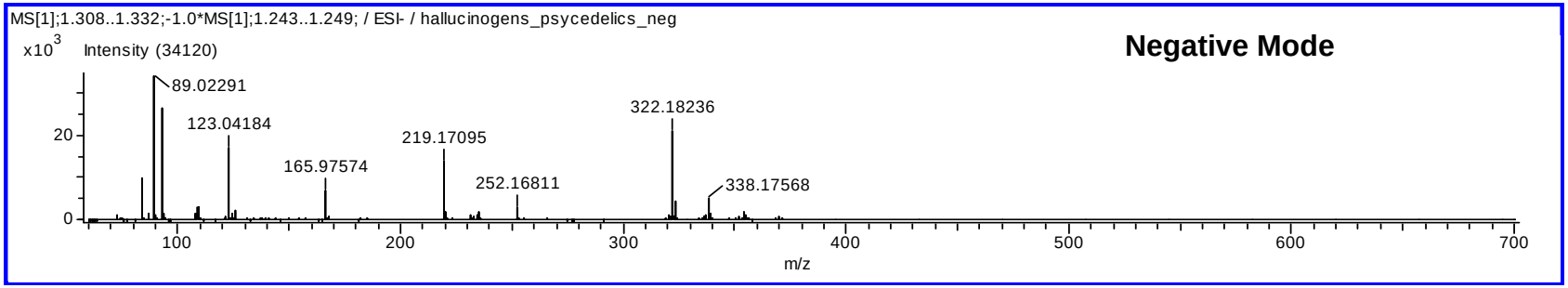




A-9. Hallucinogens/Psychedelics (continued)

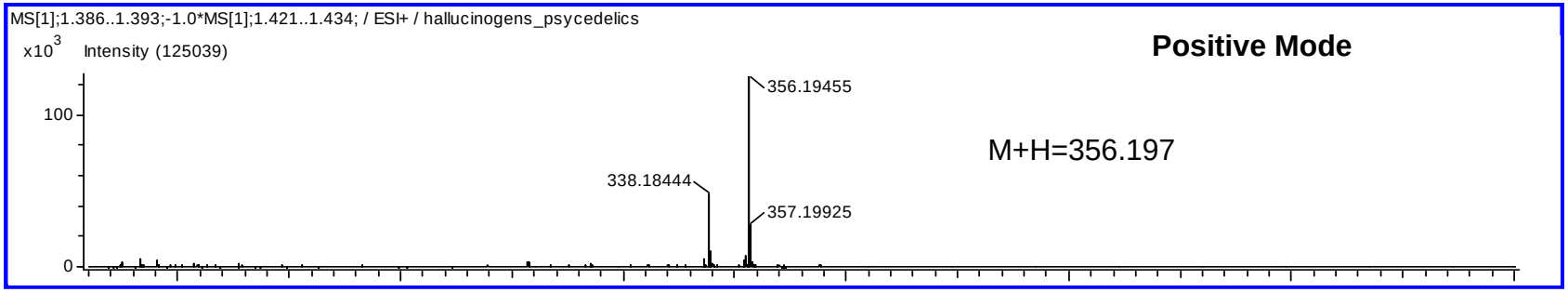
A-9.1. LSD

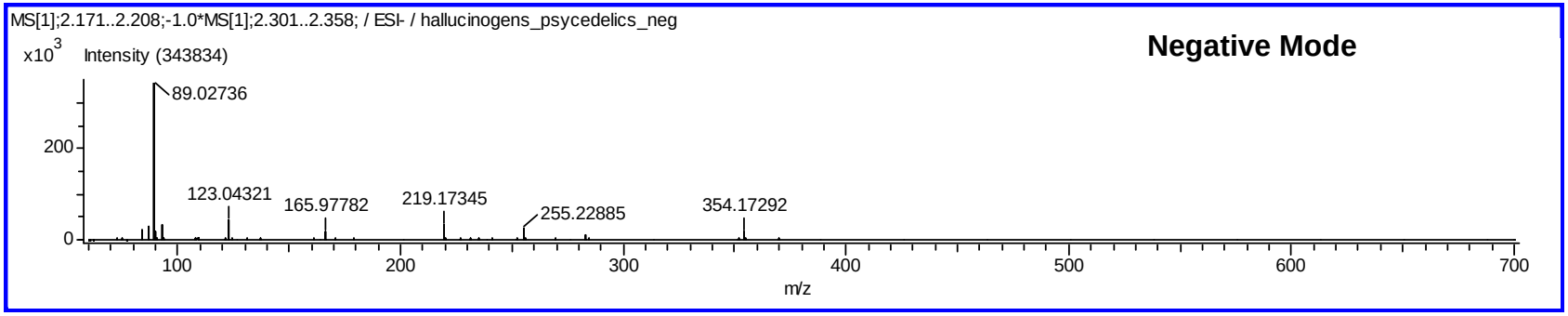




A-9. Hallucinogens/Psychedelics (continued)

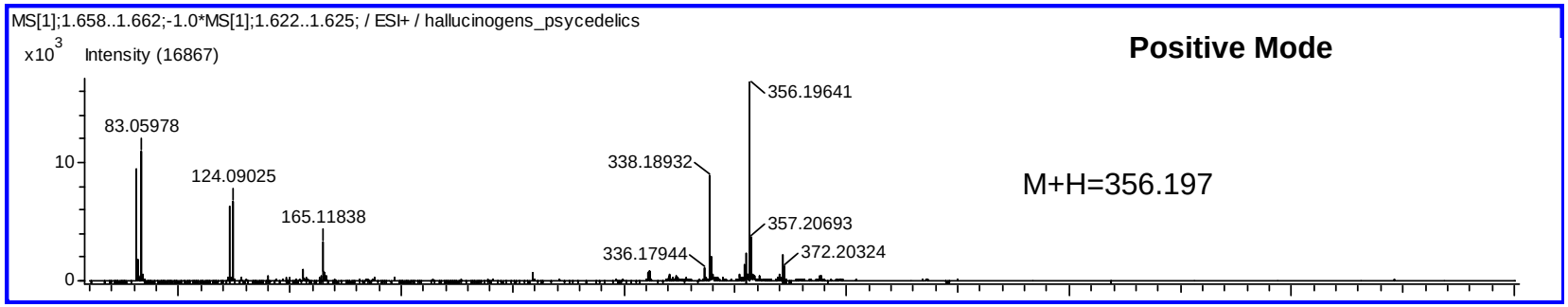
A-9.2. 2-oxo-3-hydroxy-LAMPA

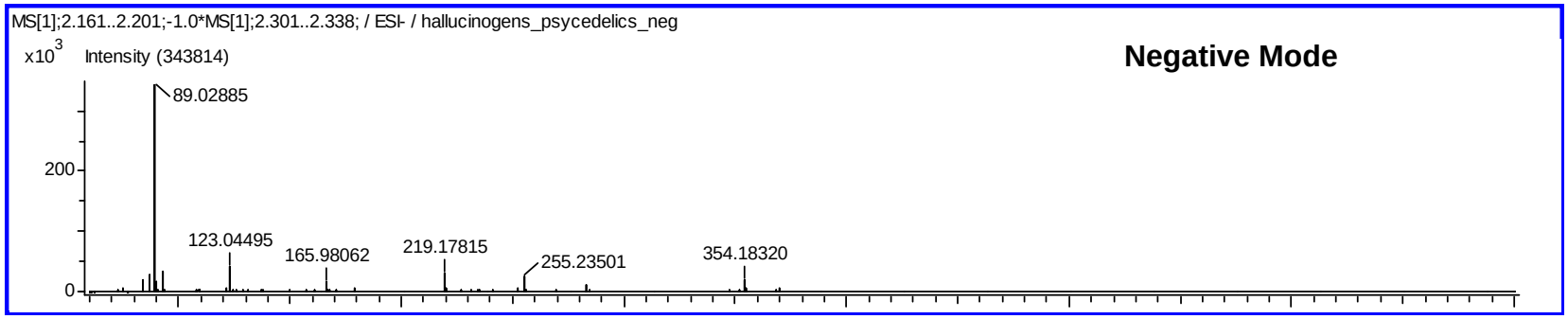




A-9. Hallucinogens/Psychedelics (continued)

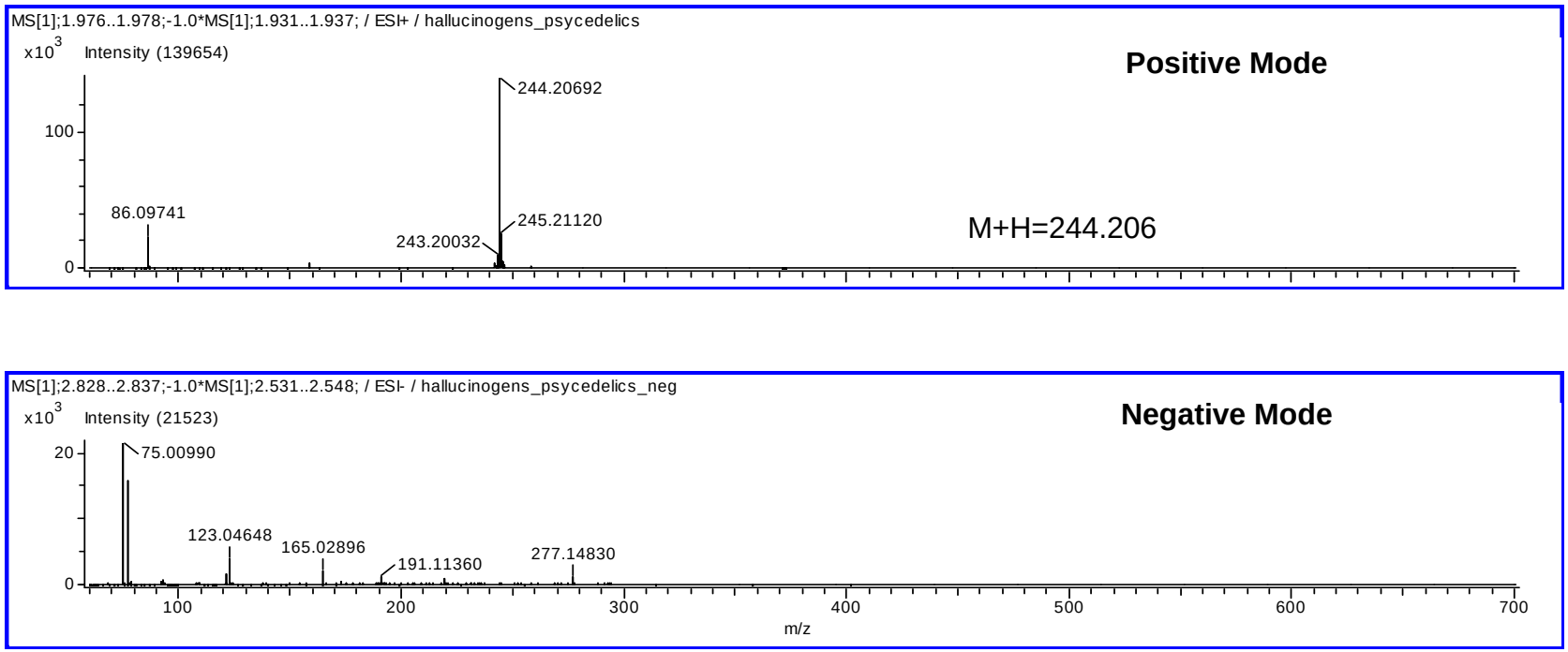
A-9.3. 2-oxo-3-hydroxy-LSD





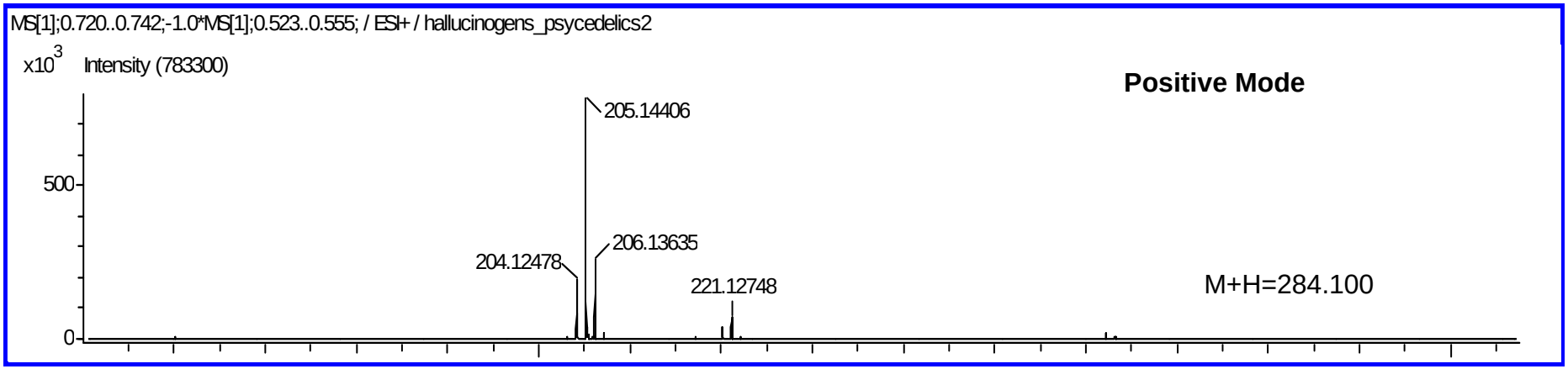
A-9. Hallucinogens/Psychedelics (continued)

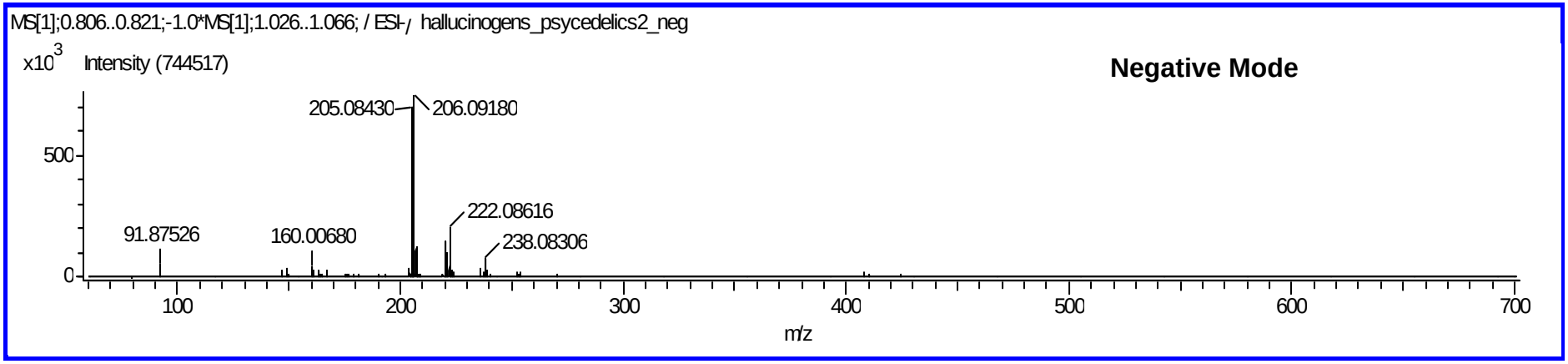
A-9.4. PCP



A-9. Hallucinogens/Psychedelics (continued)

A-9.5. Psilocybin



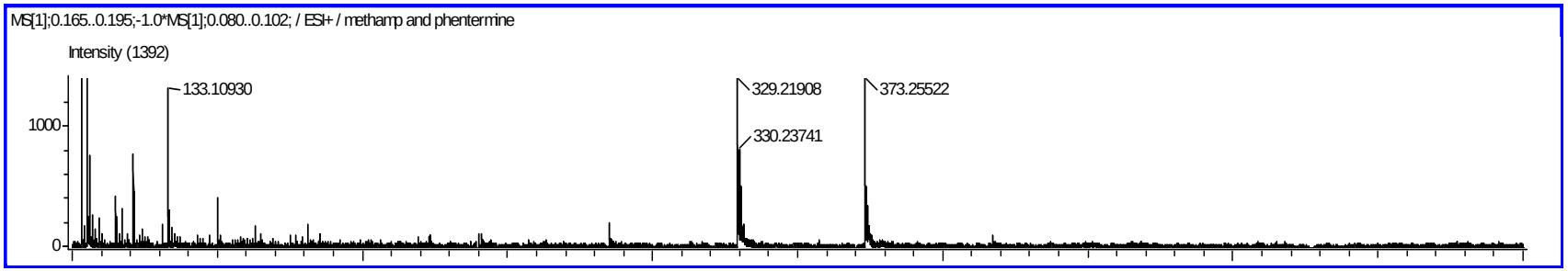


A-10. Isomers Fragmentation at 90v on O1

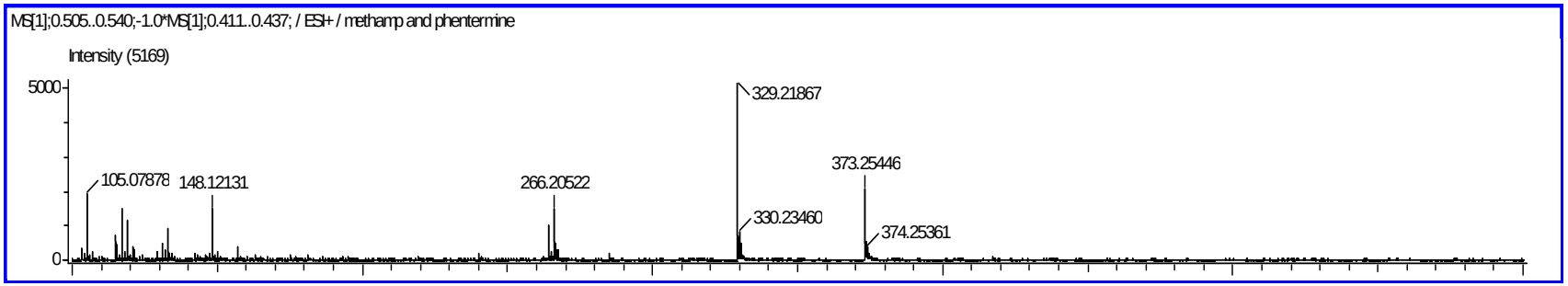
$C_{10}H_{15}N$

MW+H=150.128

A-10.1. Phentermine



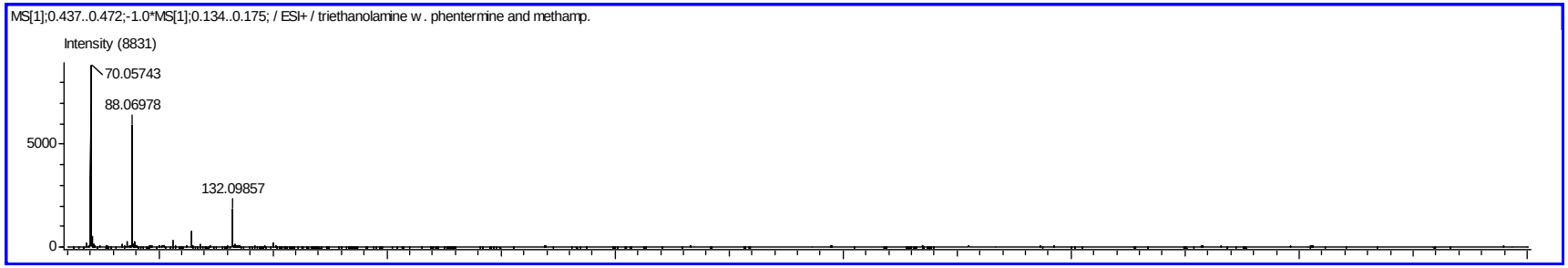
A-10.2. Methamphetamine



A-10. Isomers (continued)

$C_6H_{15}NO_3$
MW+H=150.105

A-10.3. Triethanolamine

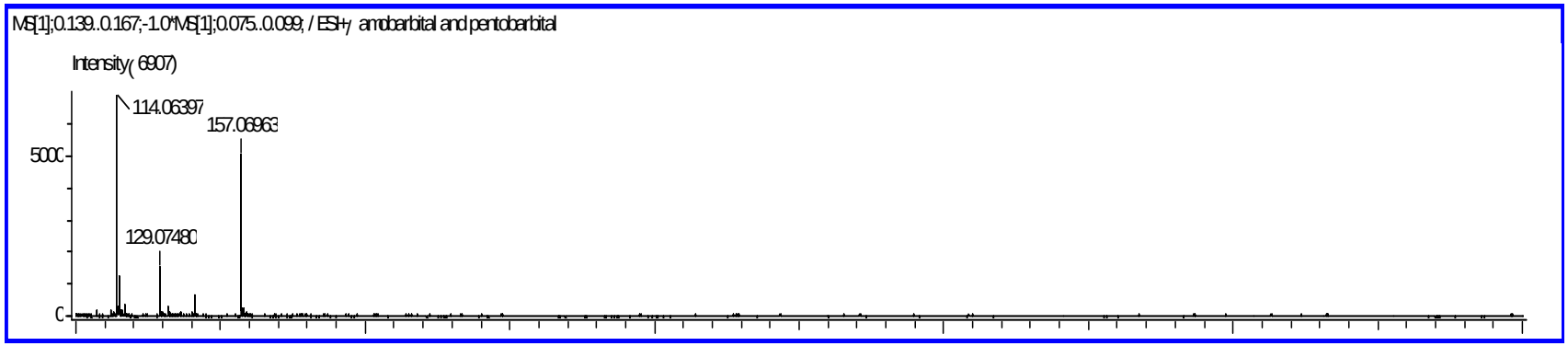


A-10. Isomers (continued)

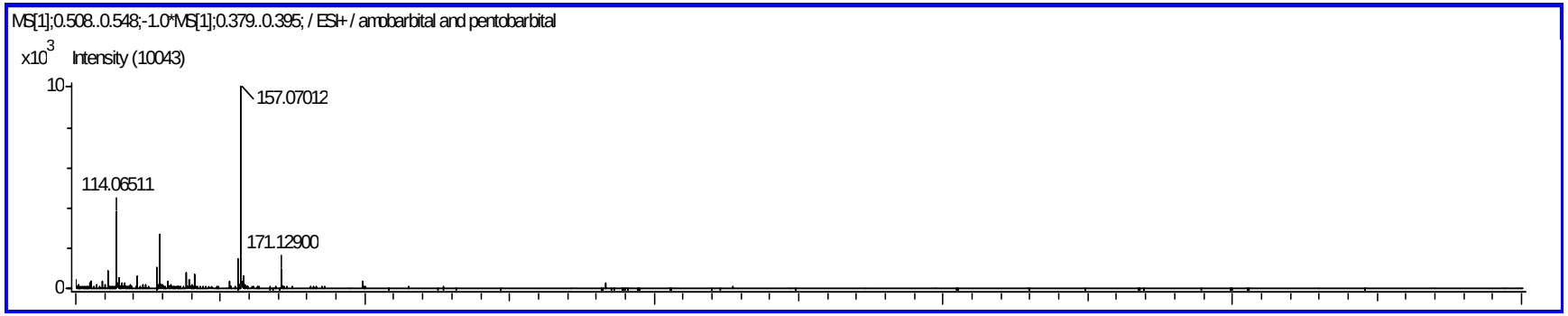
$C_{11}H_{18}N_2O_3$

MW+H=227.131

A-10.4. Pentobarbital



A-10.5. Amobarbital

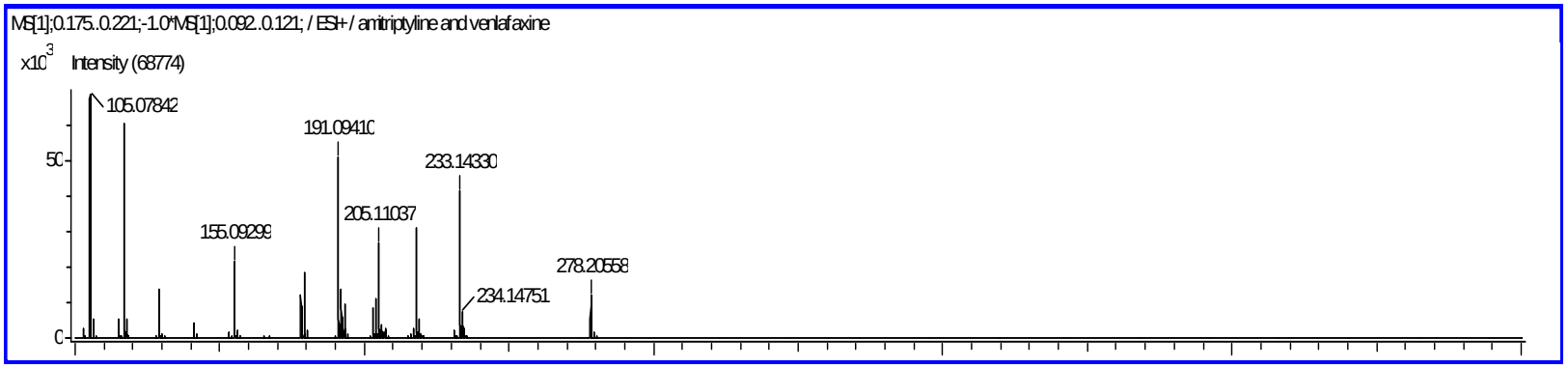


A-10. Isomers (continued)

$C_{20}H_{23}N$

MW+H=278.183

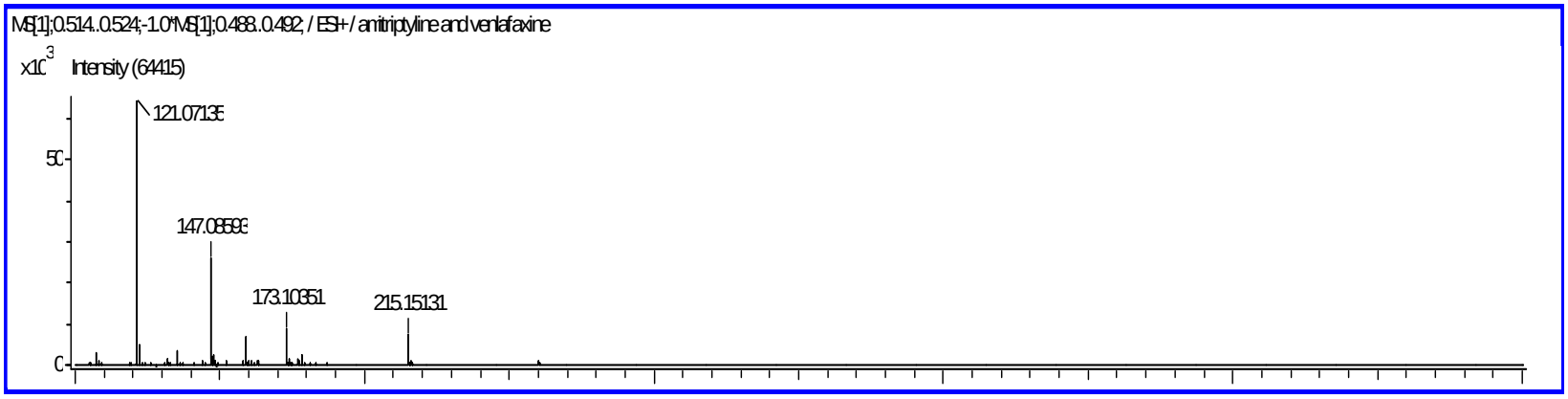
A-10.6. Amitriptyline



$C_{17}H_{27}NO_2$

MW+H=278.204

A-10.7. Venlafaxine

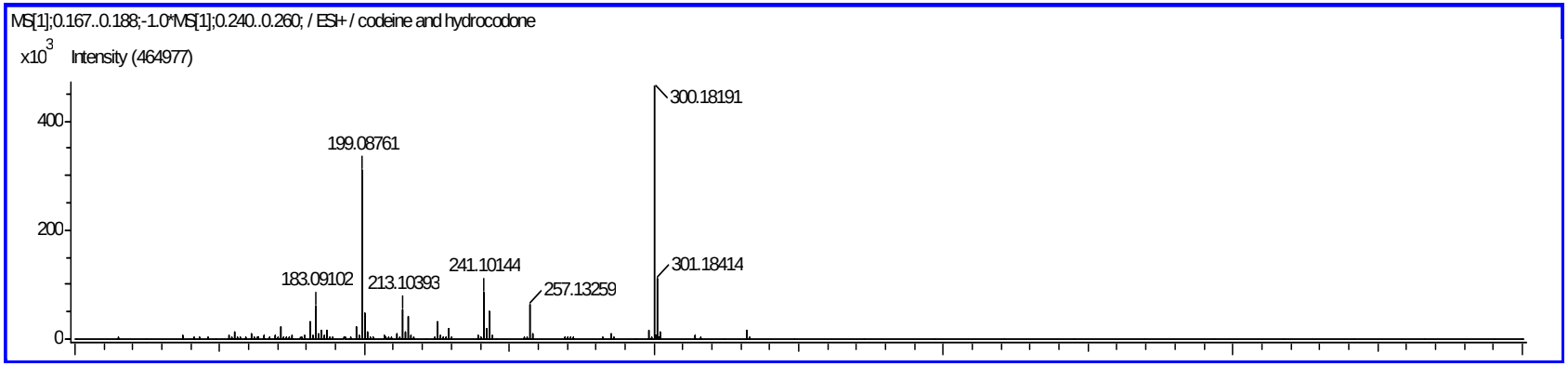


$C_{18}H_{21}NO_3$

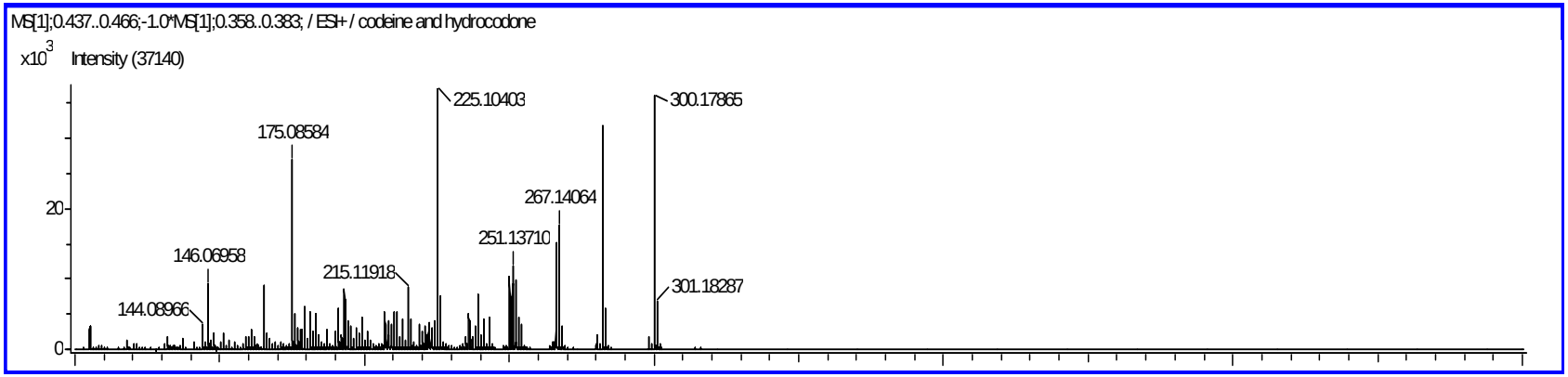
MW+H=300.152

A-10. Isomers (continued)

A-10.8. Codeine



A-10.9. Hydrocodone

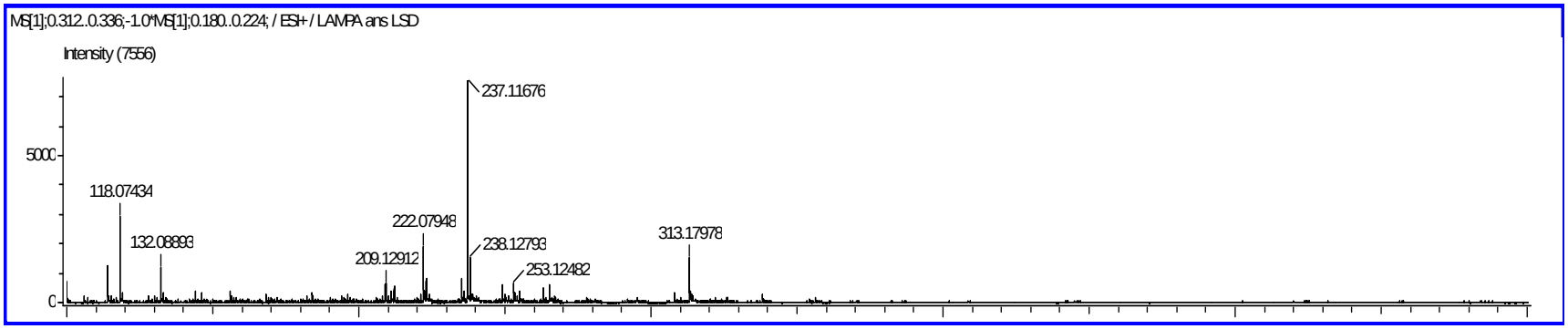


A-10. Isomers (continued)

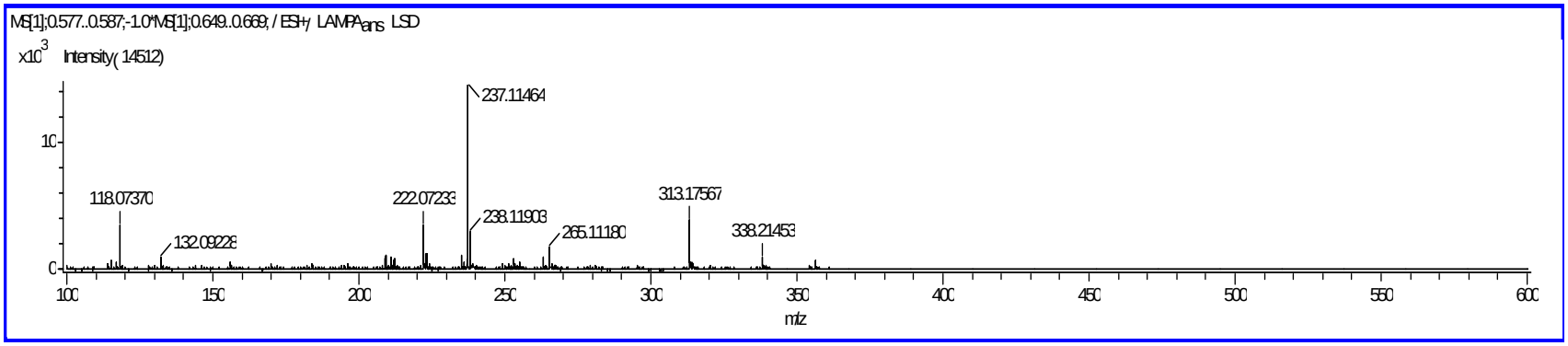
$C_{20}H_{25}N_3O_3$

MW+H=356.189

A-10.10. 2-oxo-3-hydroxy-LSD



A-10.11. 2-oxo-3-hydroxy-LAMPA

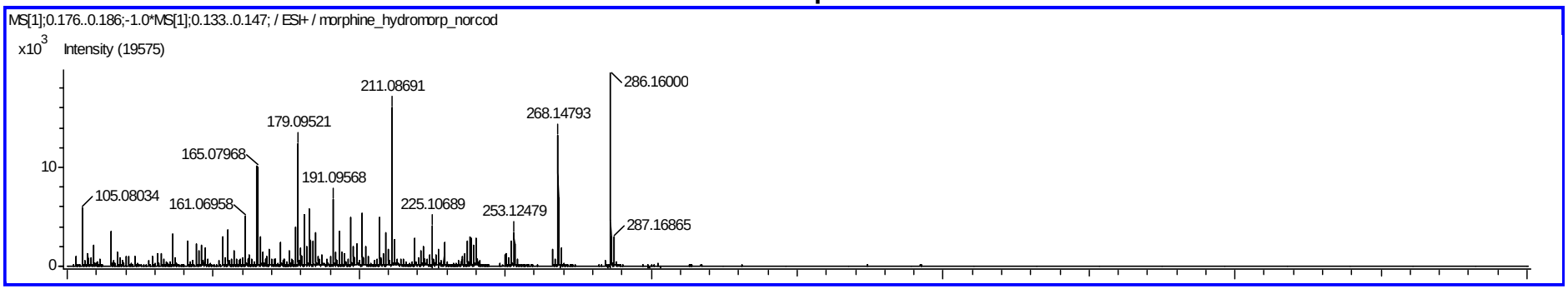


$C_{17}H_{19}NO_3$

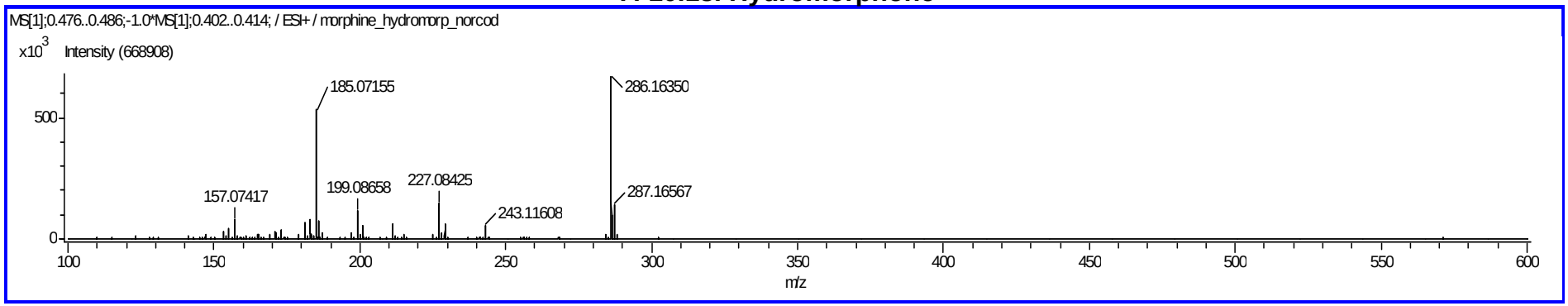
A-10. Isomers (continued)

MW+H=286.136

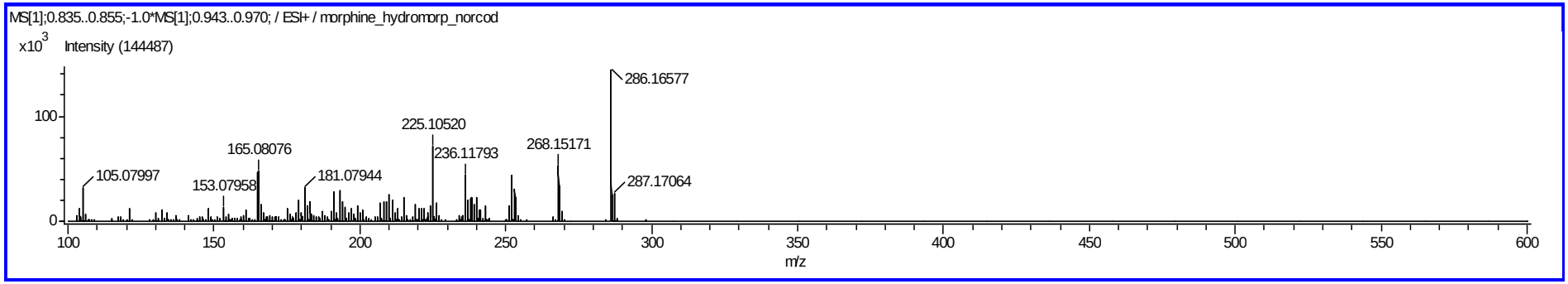
A-10.12. Morphine



A-10.13. Hydromorphone



A-10.14. Norcodeine

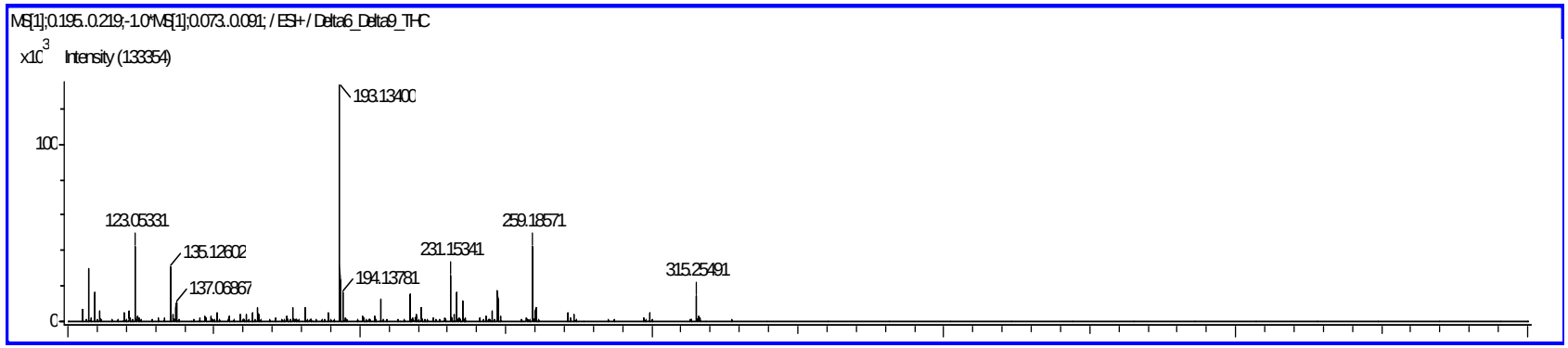


$C_{21}H_{30}O_2$

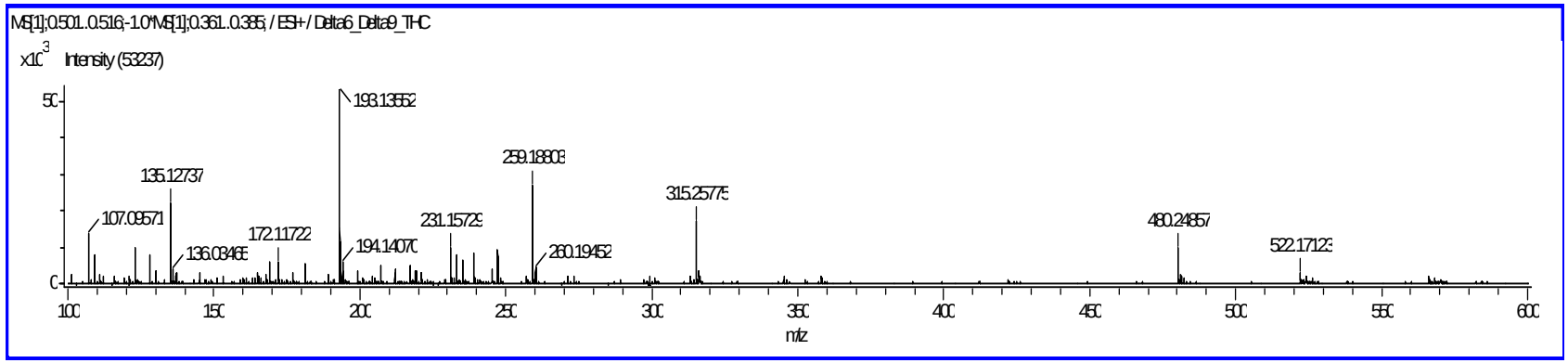
MW+H=315.224

A-10. Isomers (continued)

A-10.15. Δ9-THC



A-10.16. Δ6-THC

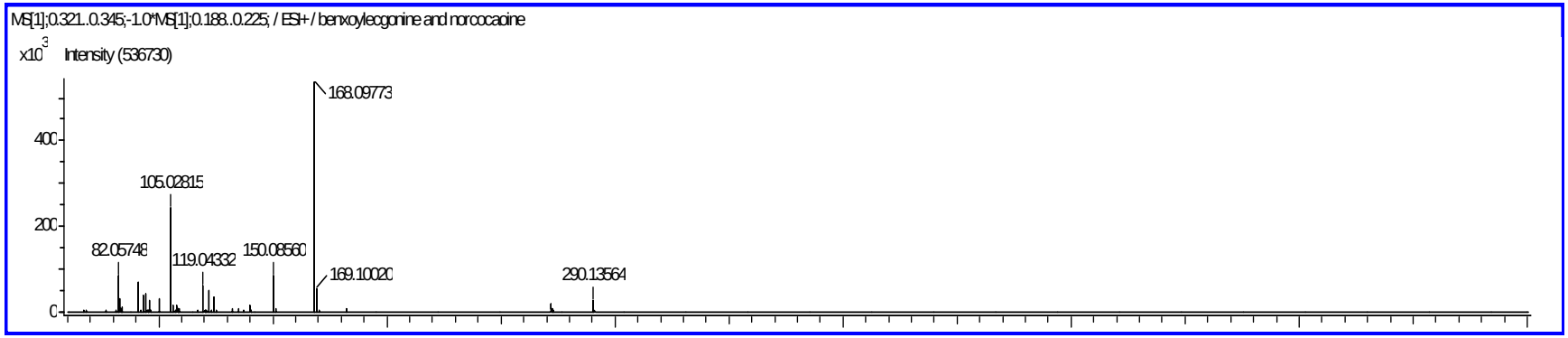


$C_{16}H_{19}NO_4$

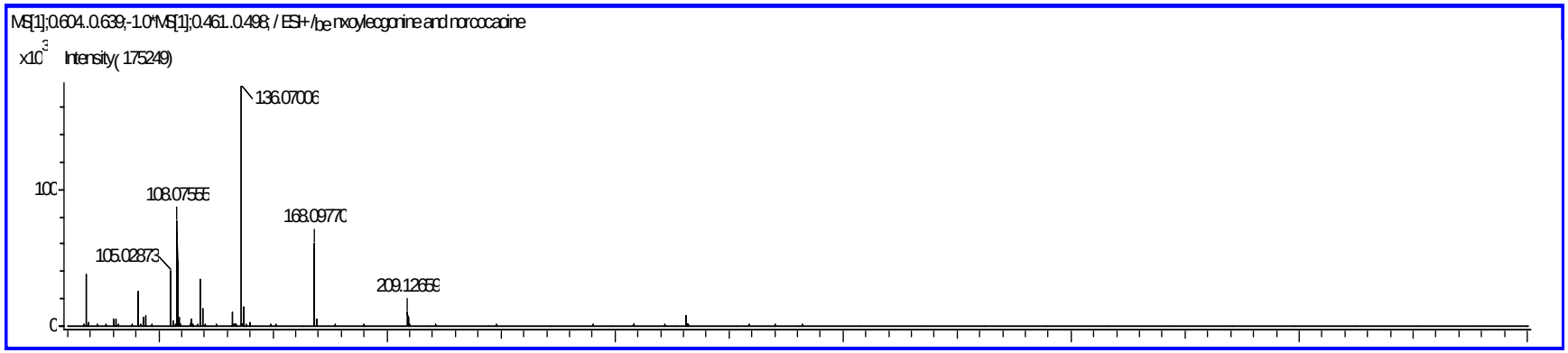
MW+H=290.131

A-10. Isomers (continued)

A-10.17. Benzoylecgonine



A-10.18. Norcocaine

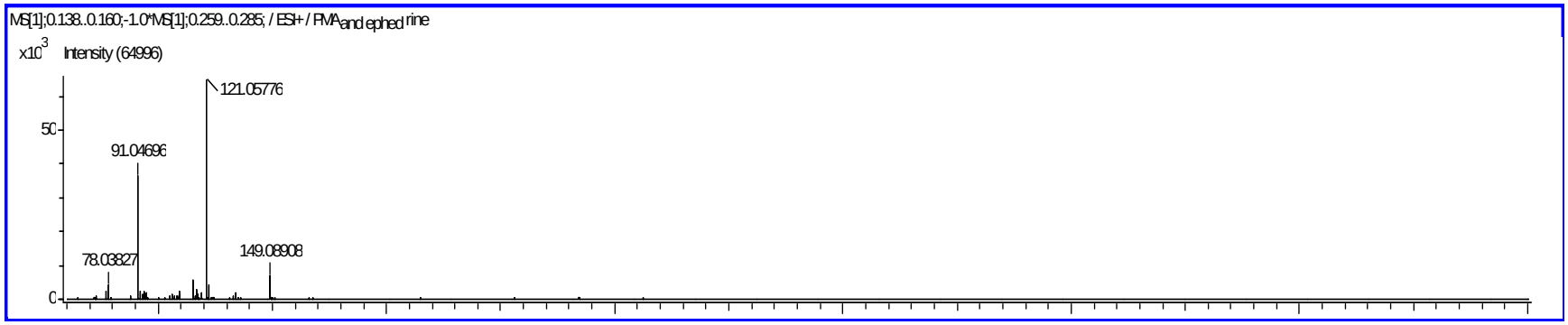


$C_{10}H_{15}NO$

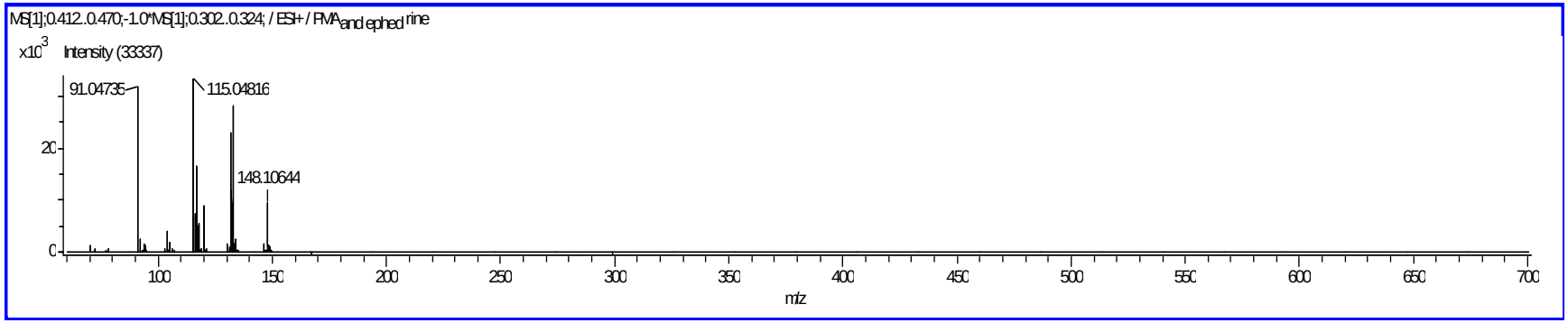
MW+H=165.115

A-10. Isomers (continued)

A-10.19. PMA



A-10.20. Ephedrine

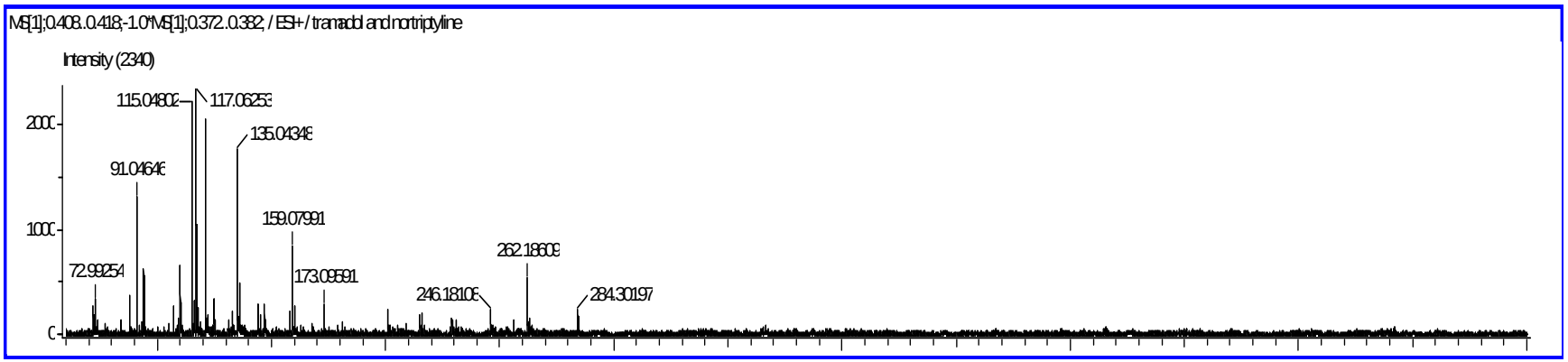


A-10. Isomers (continued)

$C_{16}H_{25}NO_2$

MW+H=264.188

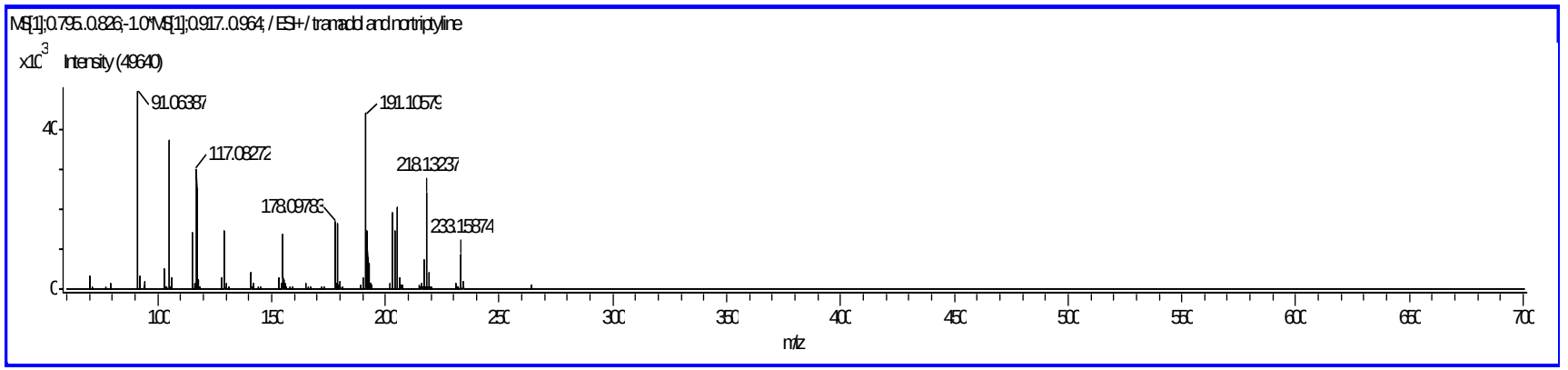
A-10.21. Tramadol



$C_{19}H_{21}N$

MW+H=264.167

A-10.22. Nortriptyline

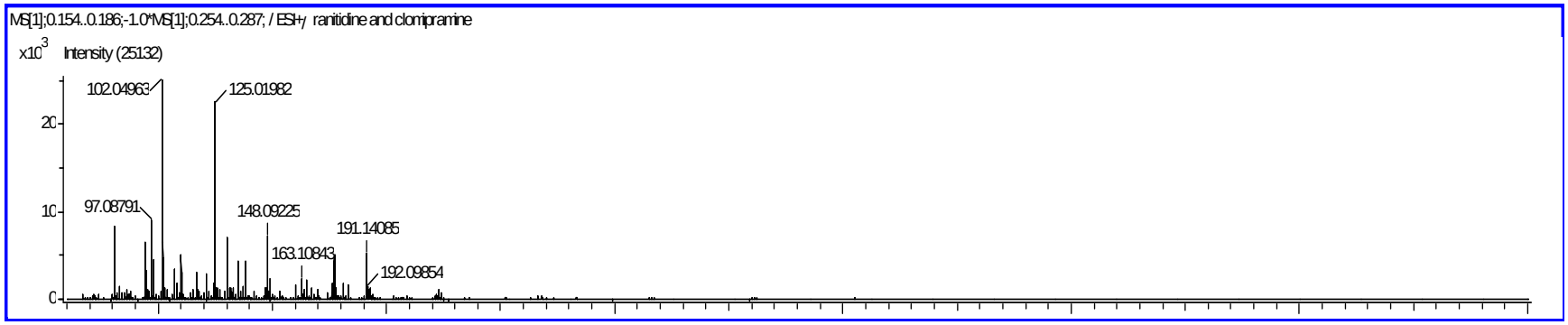


A-10. Isomers (continued)

$C_{13}H_{22}N_4O_3S$

MW+H=315.141

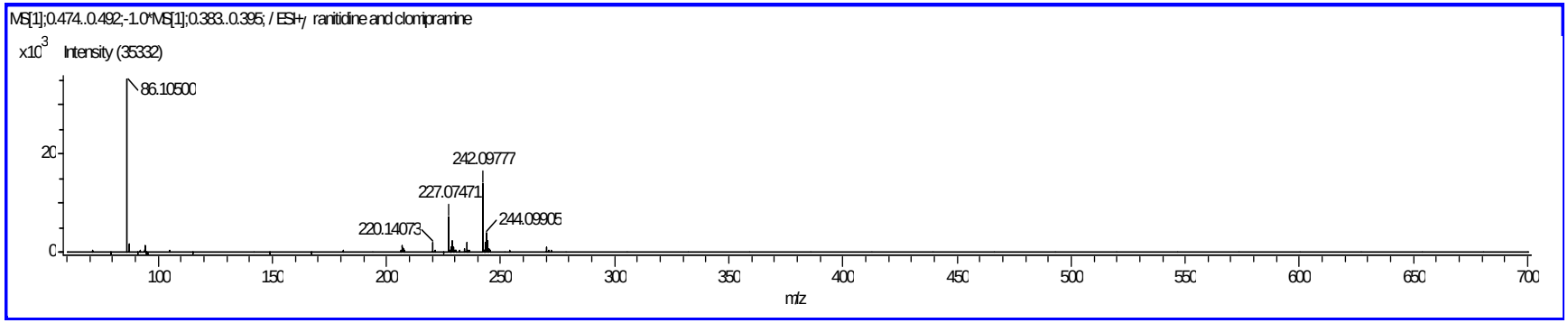
A-10.23. Ranitidine



$C_{19}H_{23}ClN_2$

MW+H=315.154

A-10.24. Chlomipramine

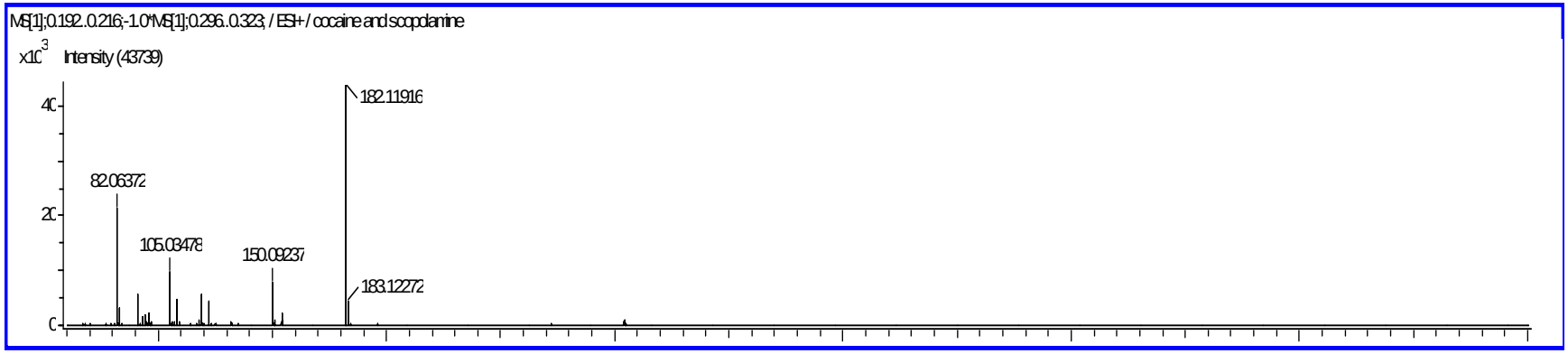


A-10. Isomers (continued)

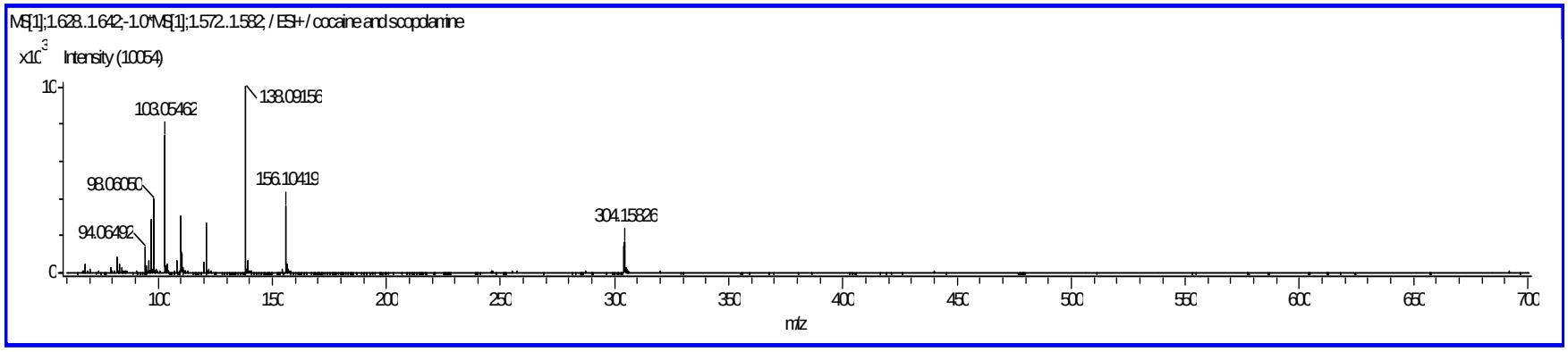
$C_{17}H_{21}NO_4$

MW+H=304.147

A-10.25. Cocaine



A-10.26. Scopolamine

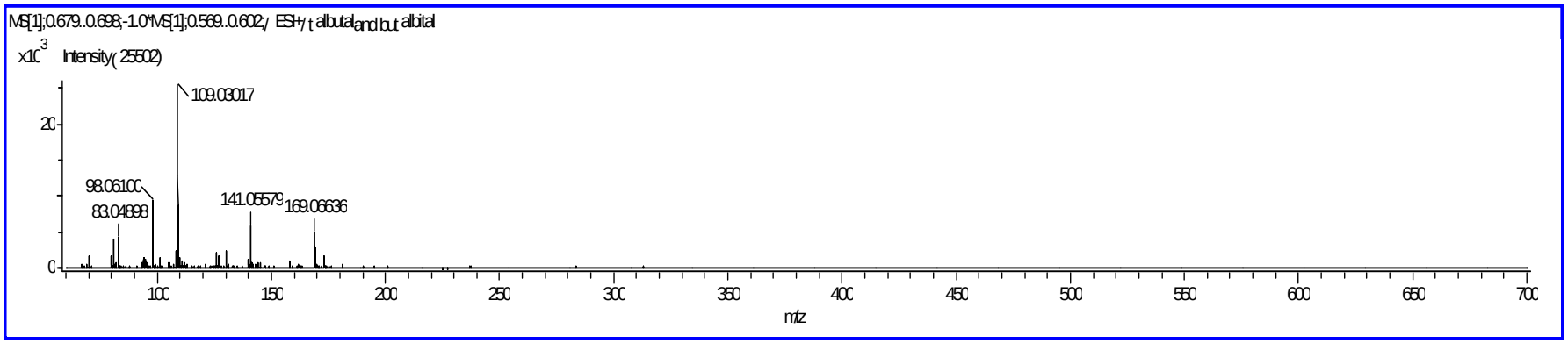


$C_{11}H_{16}N_2O_4$

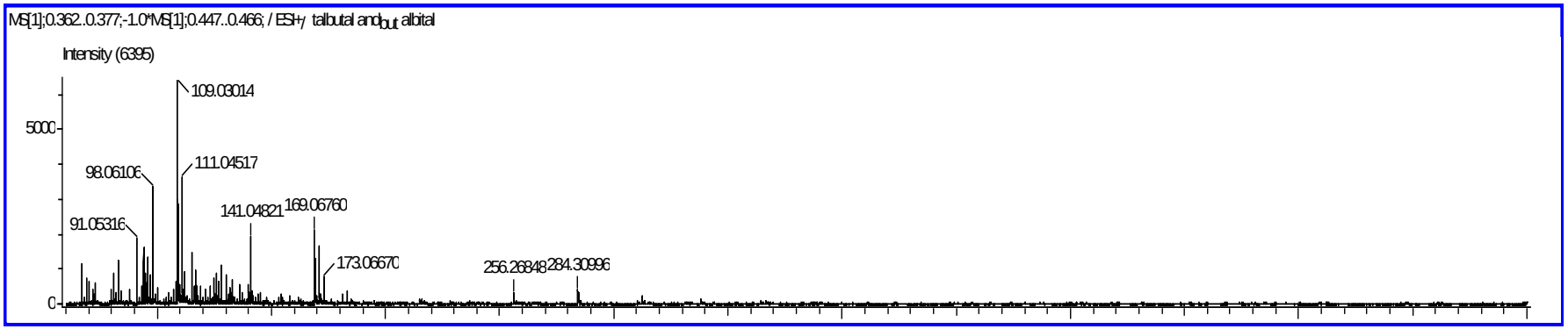
MW+H=225.116

A-10. Isomers (continued)

A-10.27. Talbutal



A-10.28. Butalbital

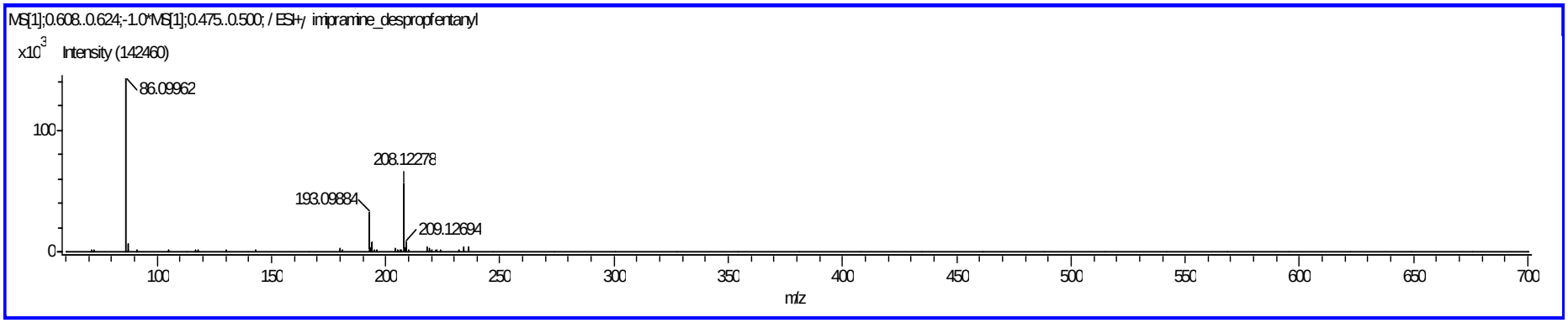


A-10. Isomers (continued)

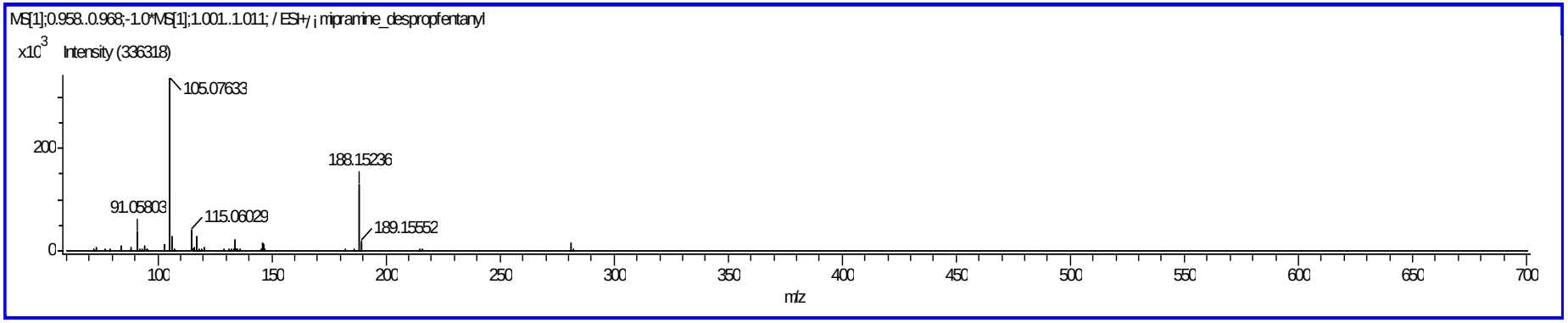
$C_{19}H_{24}N_2$

MW+H=281.193

A-10.29. Imipramine



A-10.30. Despropionyl Fentanyl

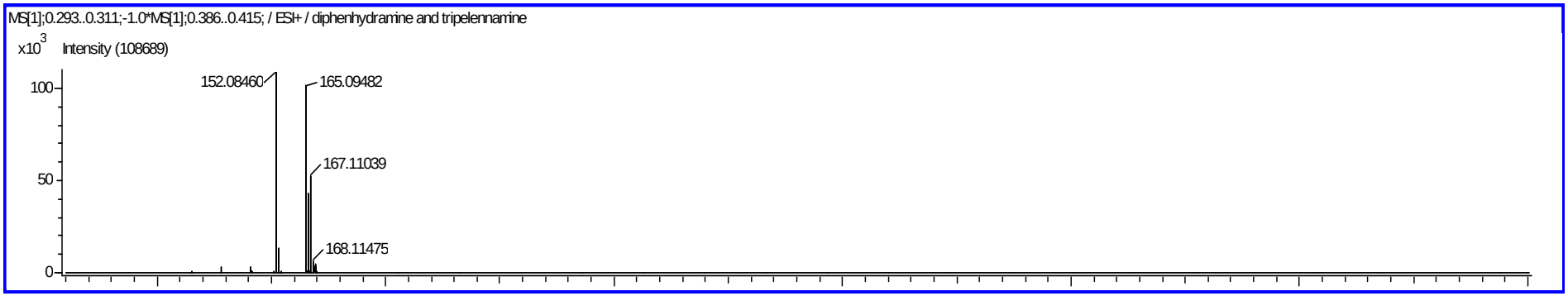


A-10. Isomers (continued)

$C_{17}H_{21}NO$

MW+H=255.162

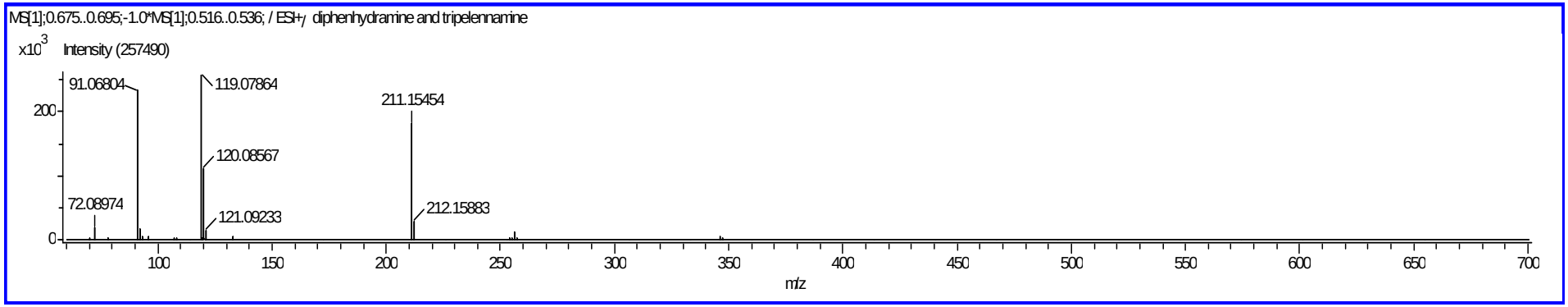
A-10.31. Diphenhydramine



$C_{16}H_{21}N_3$

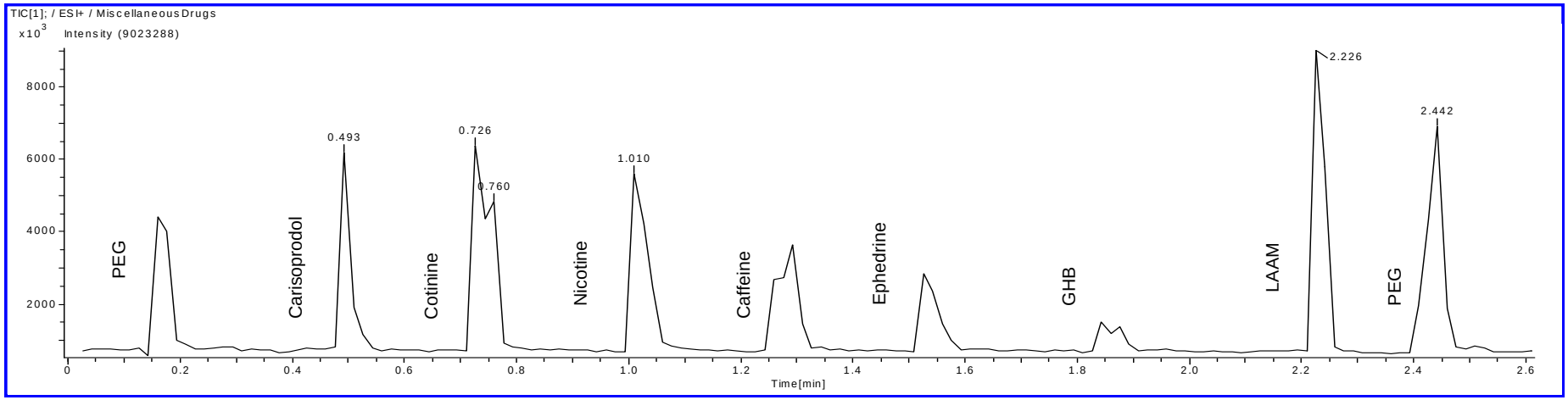
MW+H=255.173

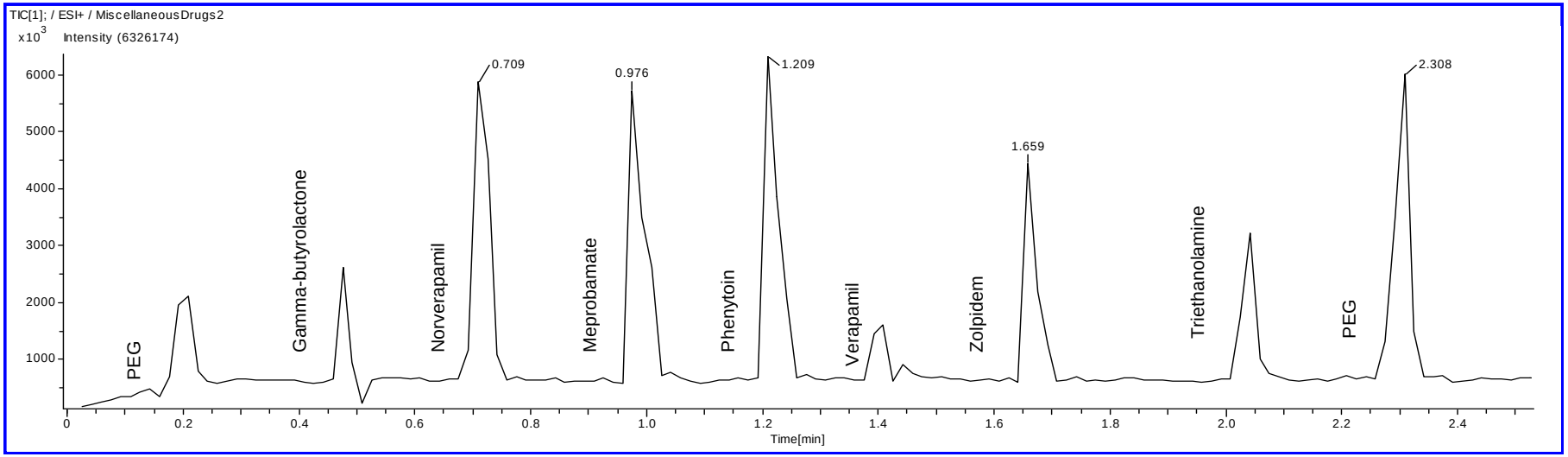
A-10.32. Tripelennamine



A-11. Miscellaneous Drugs (4/20/07)

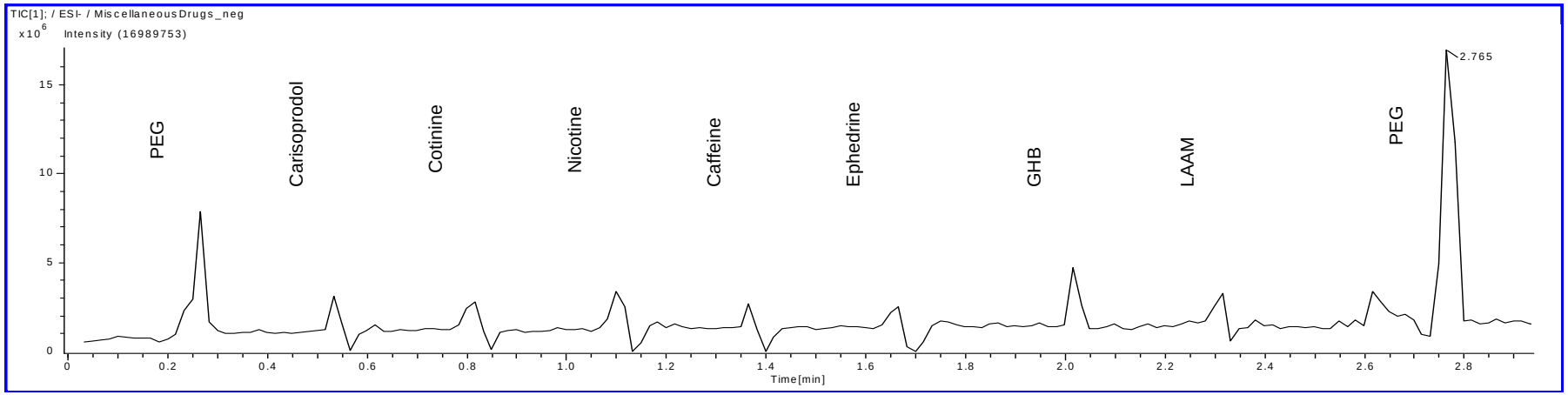
Positive Mode

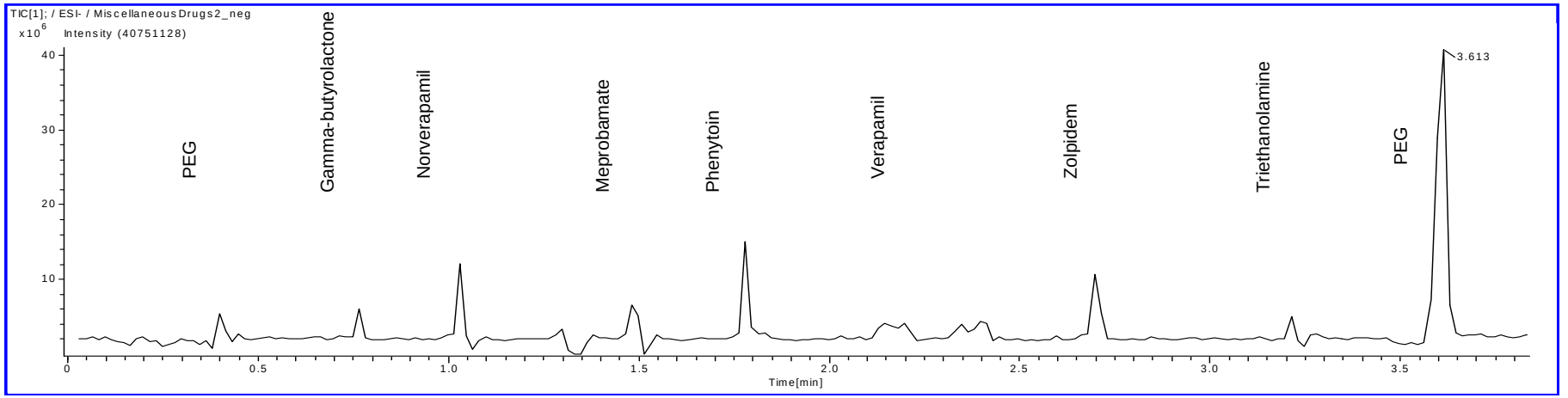




A-11. Miscellaneous Drugs (4/20/07) (continued)

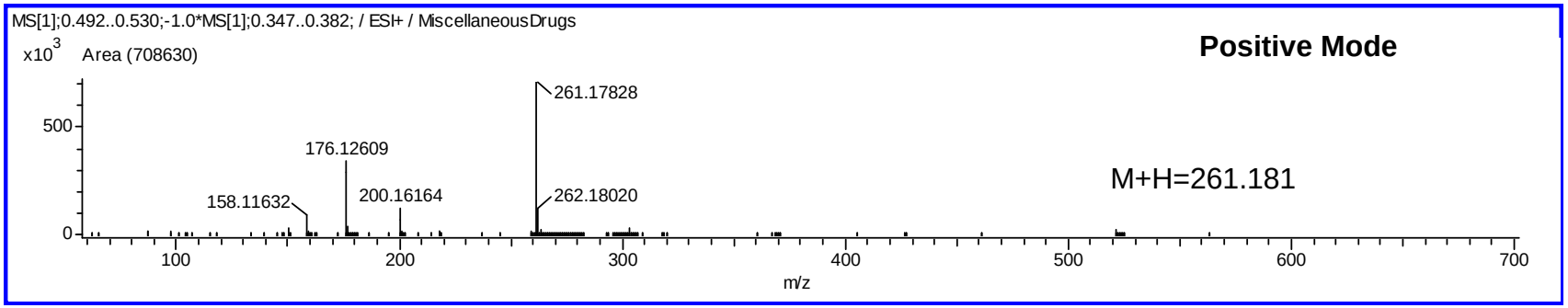
Negative Mode

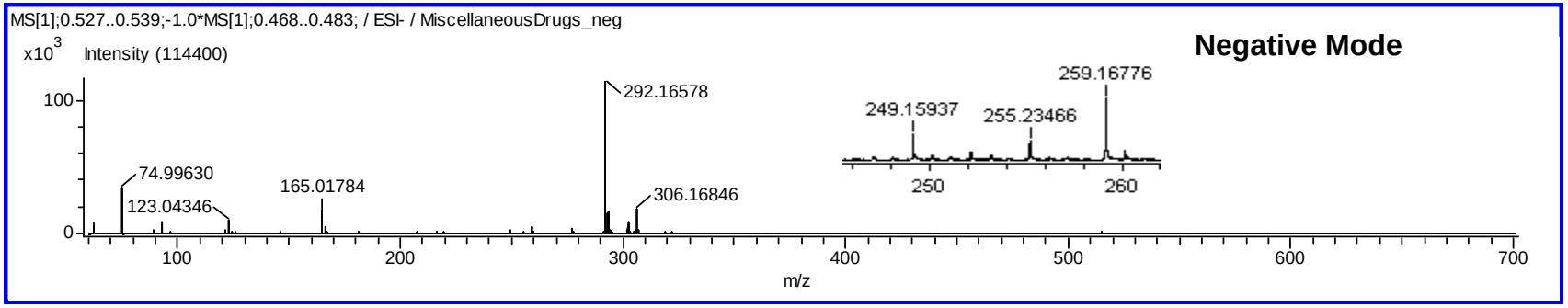




A-11. Miscellaneous Drugs (continued)

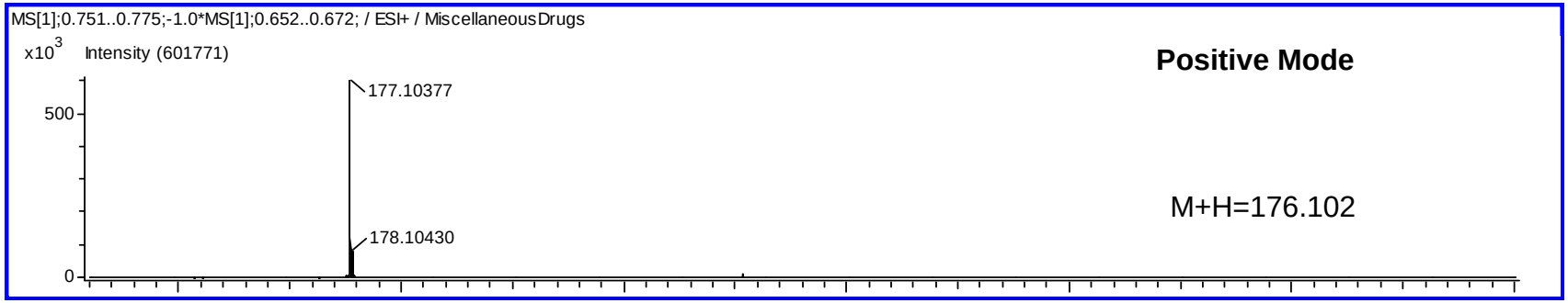
A-11.1. Carisoprodol

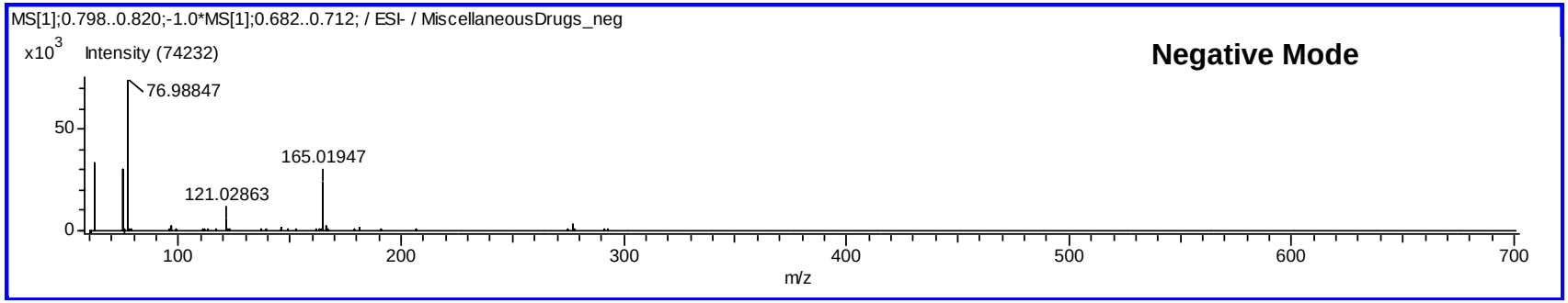




A-11. Miscellaneous Drugs (continued)

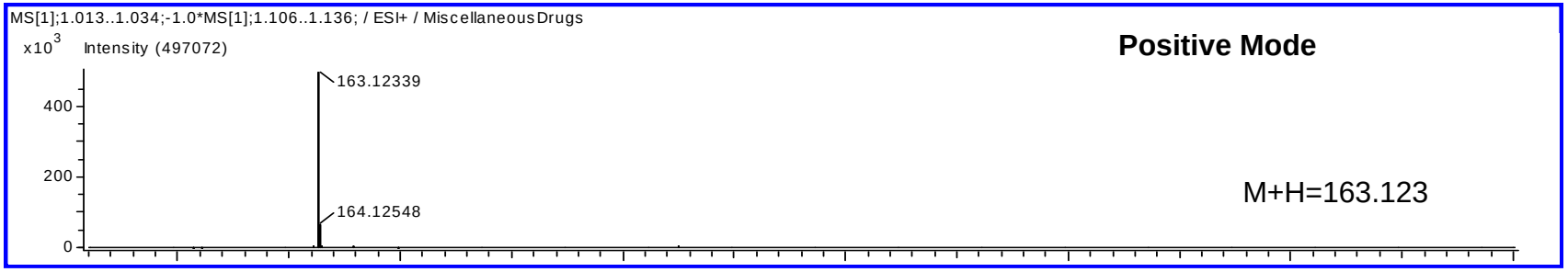
A-11.2. Cotinine

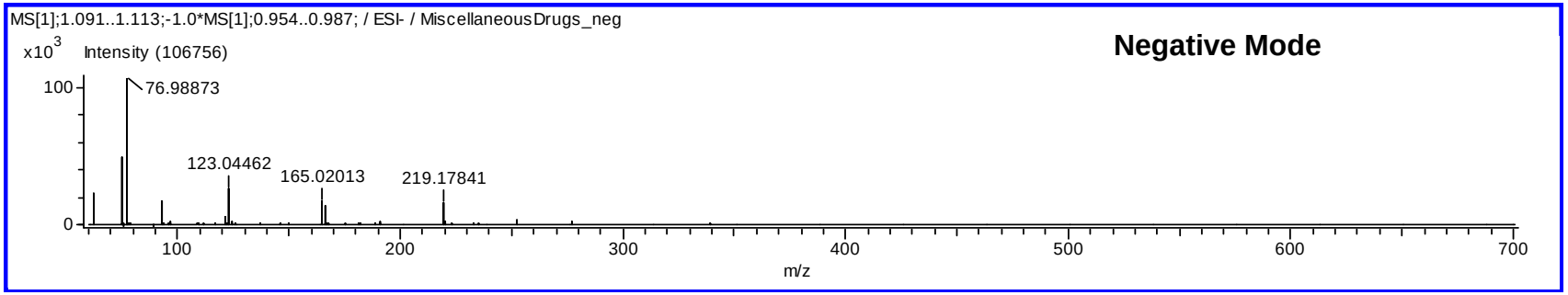




A-11. Miscellaneous Drugs (continued)

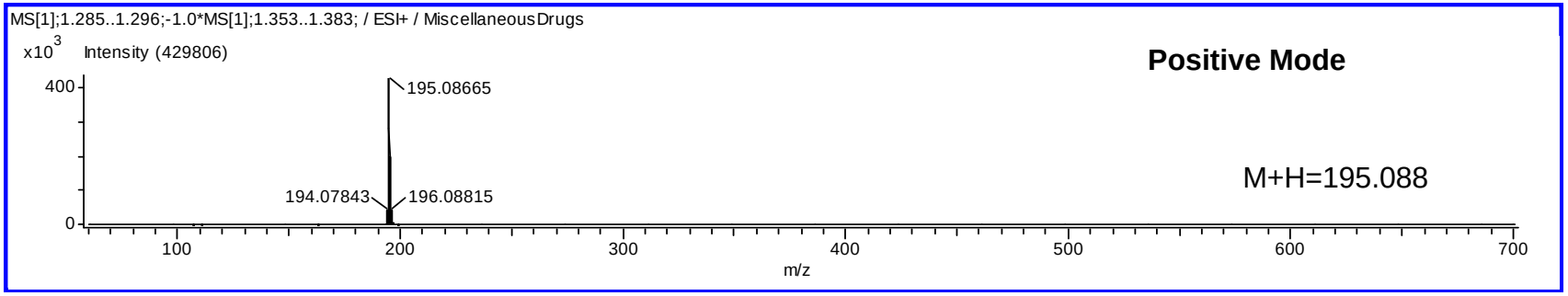
A-11.3. Nicotine

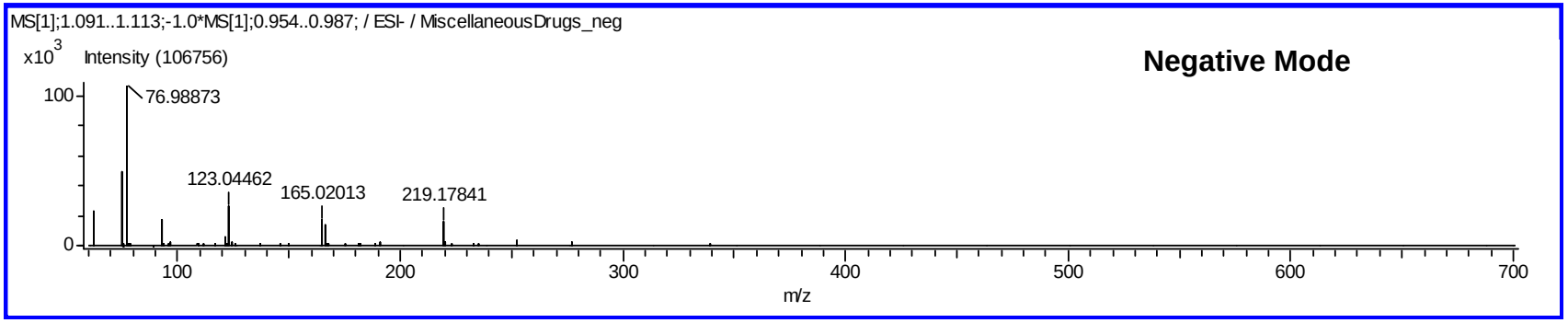




A-11. Miscellaneous Drugs (continued)

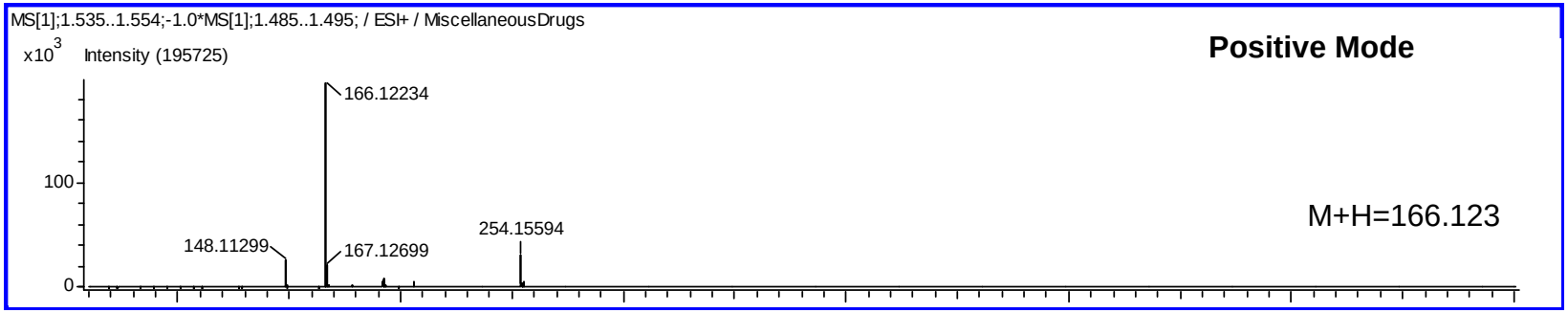
A-11.4. Caffeine

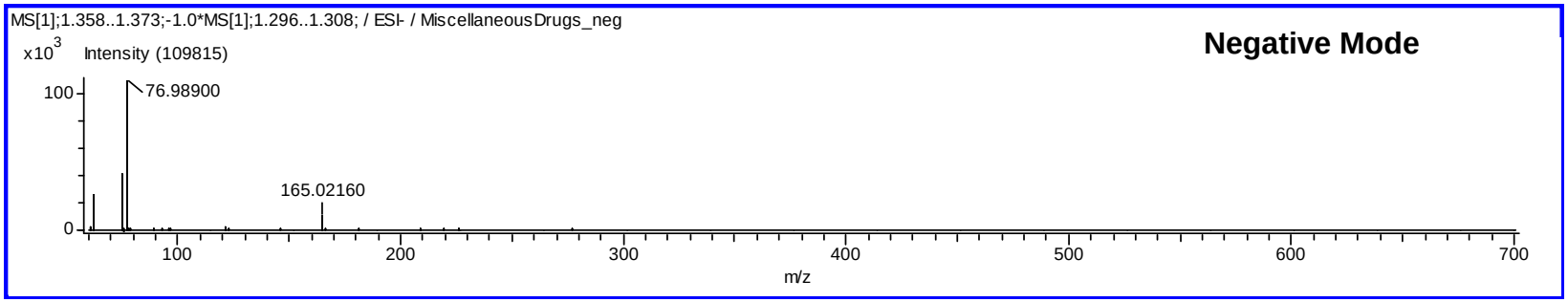




A-11. Miscellaneous Drugs (continued)

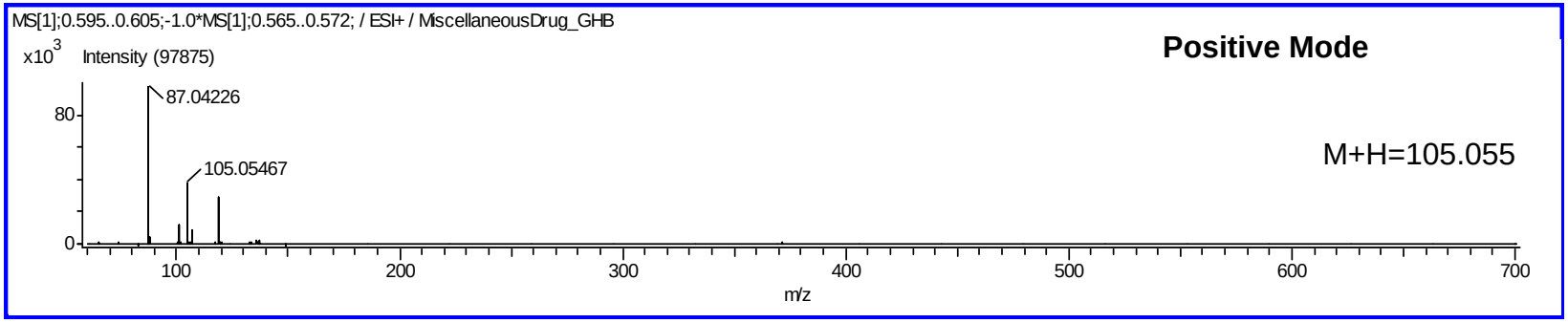
A-11.5. Ephedrine

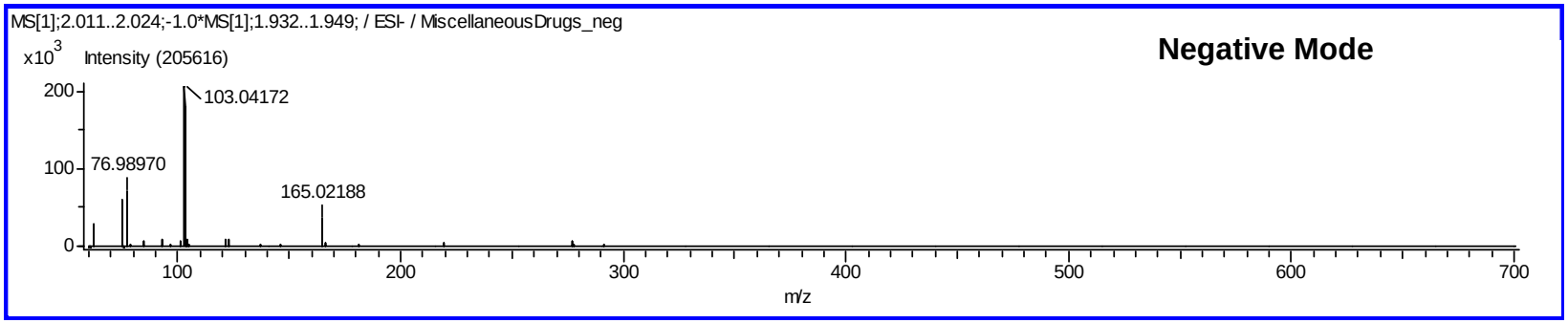




A-11. Miscellaneous Drugs (continued)

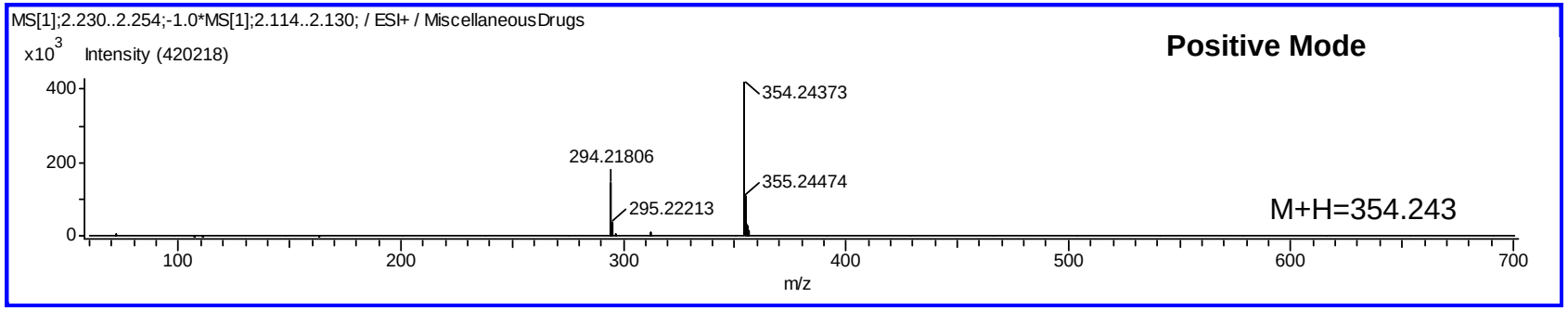
A-11.6. GHB

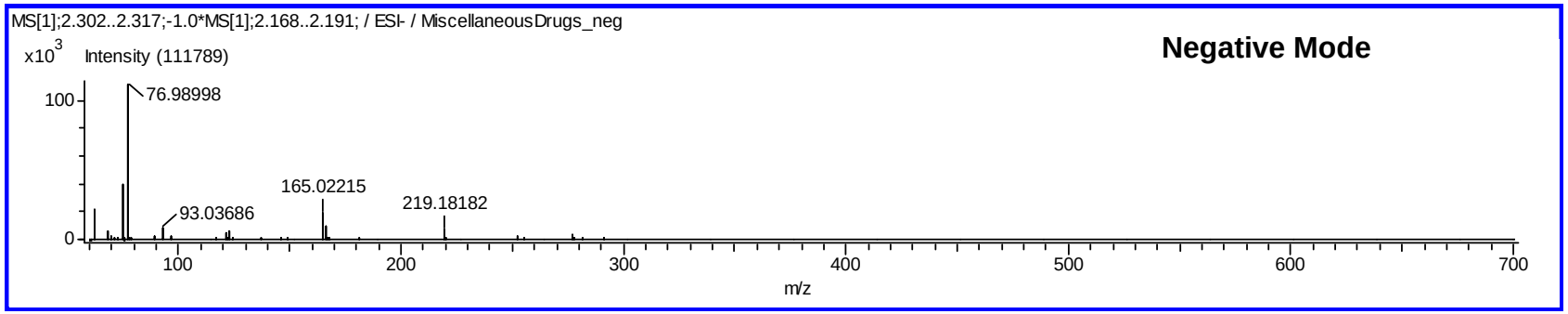




A-11. Miscellaneous Drugs (continued)

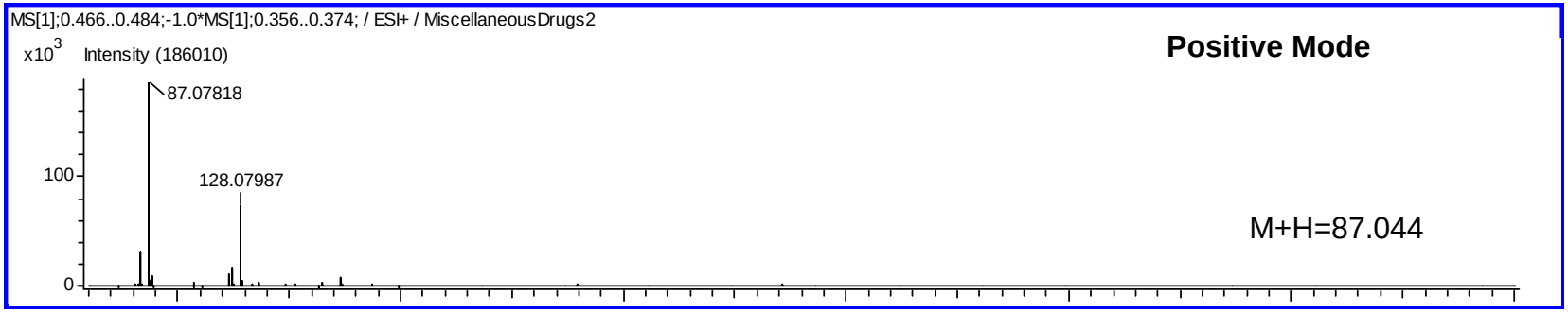
A-11.7. LAAM

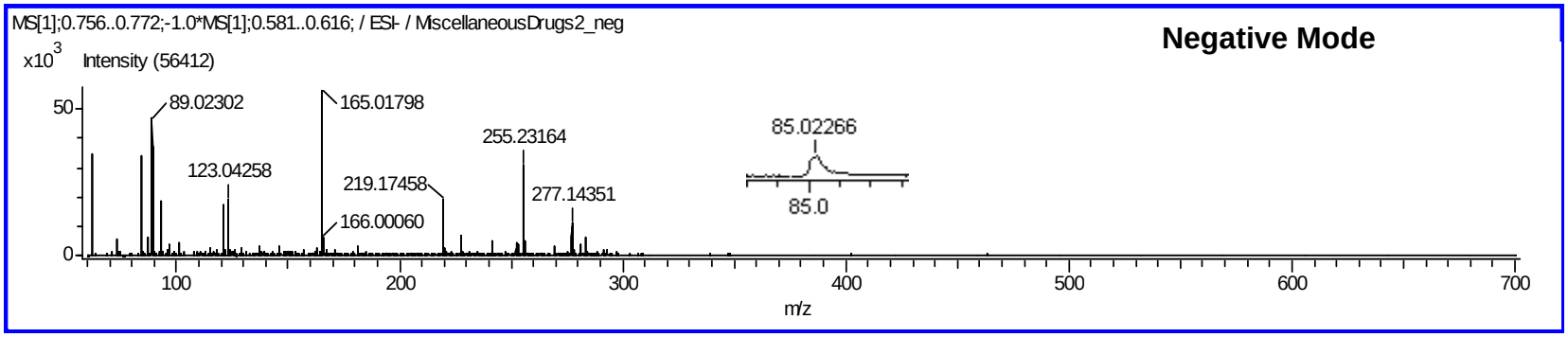




A-11. Miscellaneous Drugs (continued)

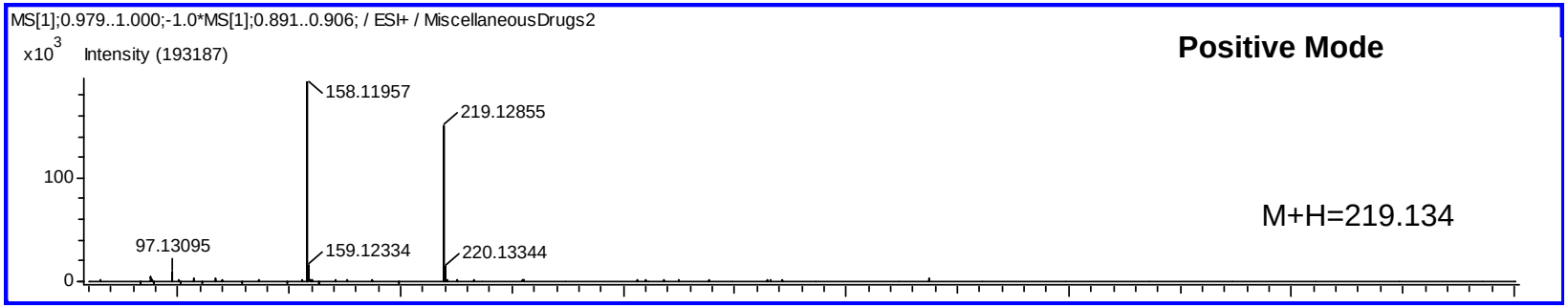
A-11.8. Gamma-butyrolactone

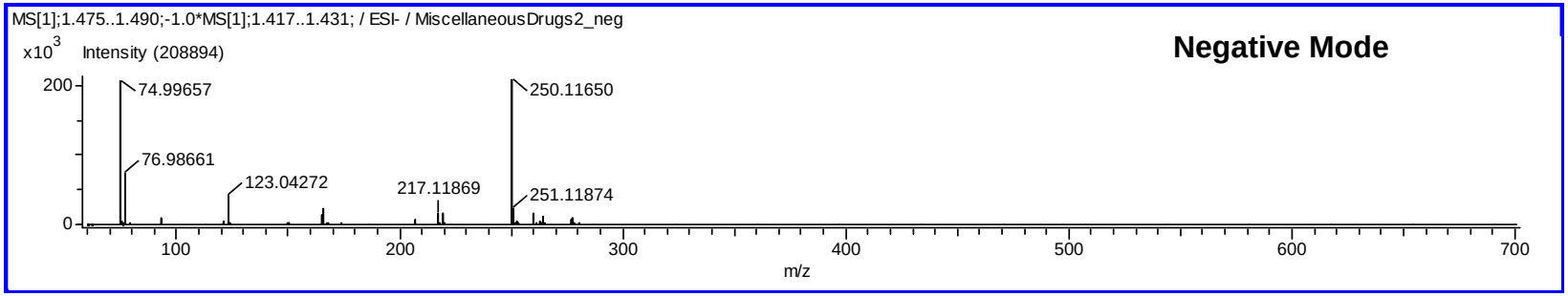




A-11. Miscellaneous Drugs (continued)

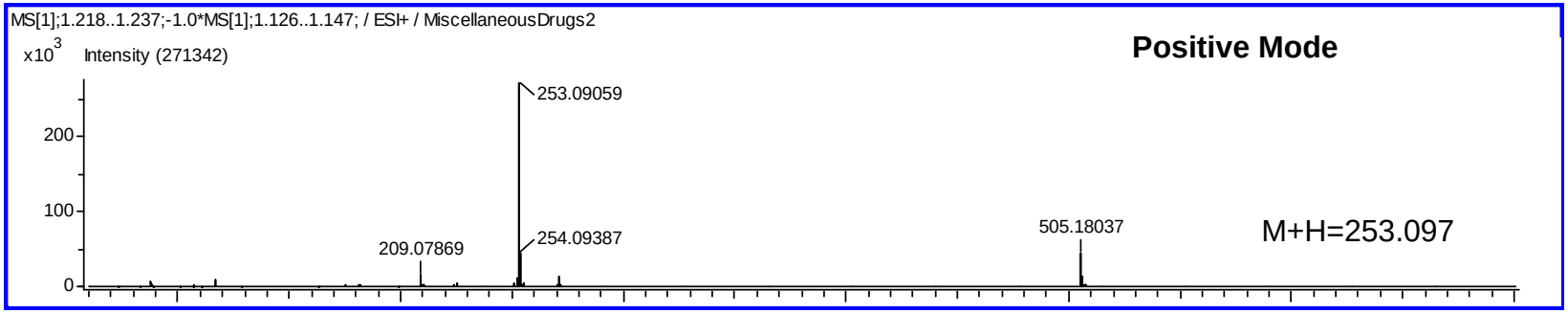
A-11.9. Meprobamate

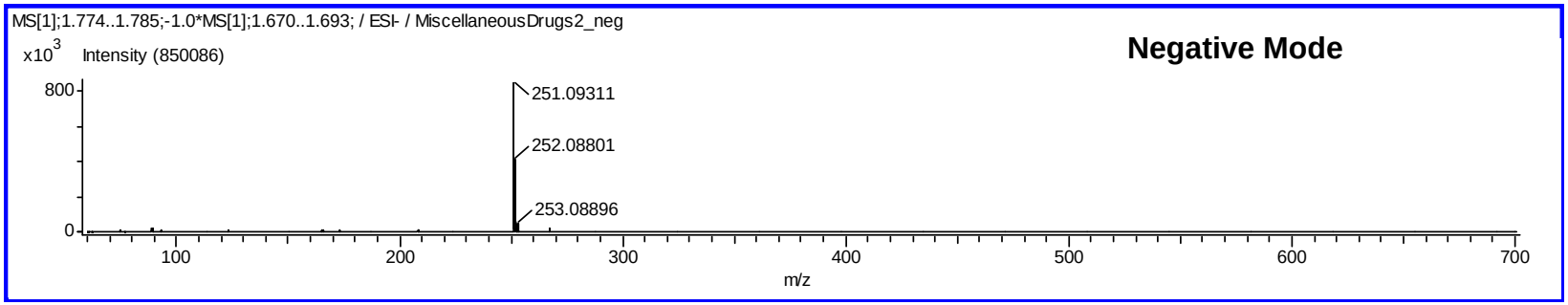




A-11. Miscellaneous Drugs (continued)

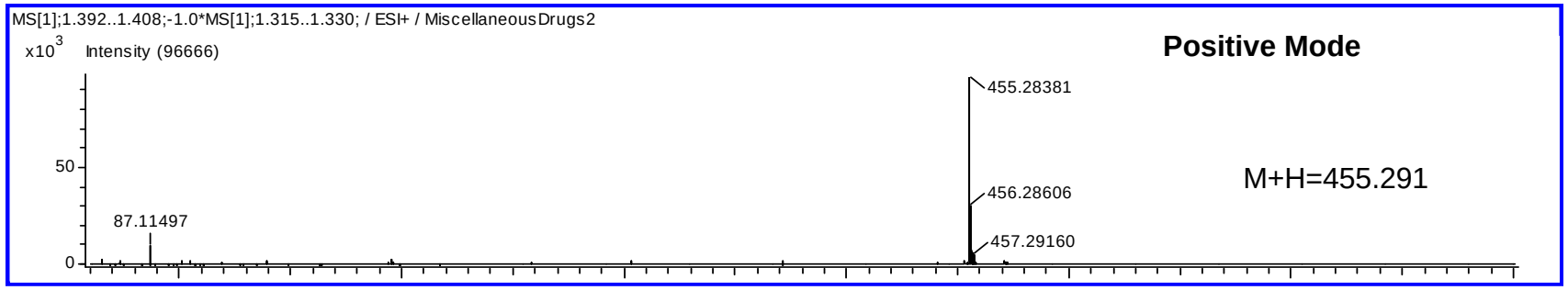
A-11.10. Phenytoin

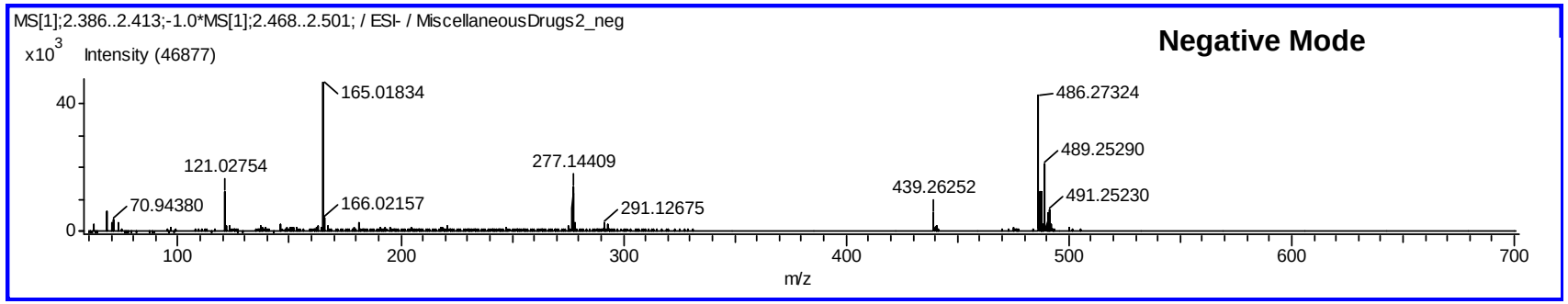




A-11. Miscellaneous Drugs (continued)

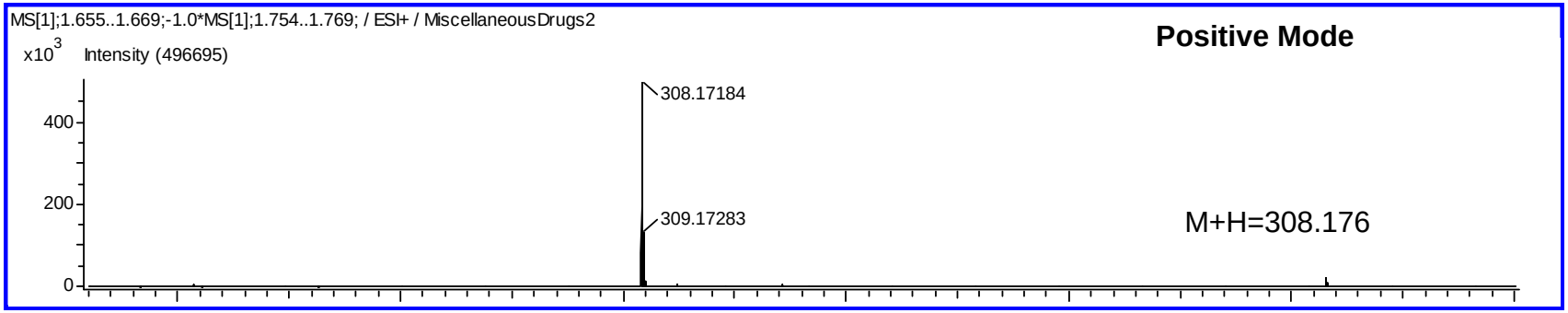
A-11.11. Verapamil

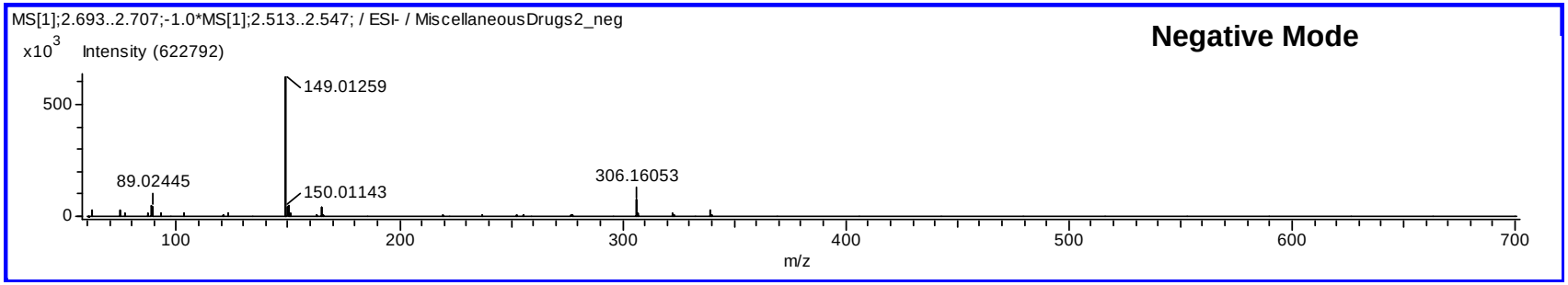




A-11. Miscellaneous Drugs (continued)

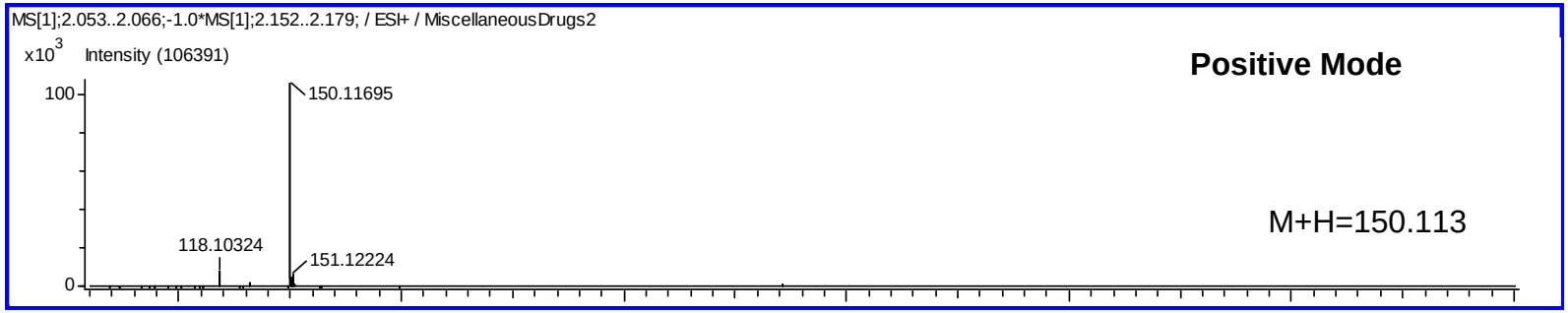
A-11.12. Zolpidem

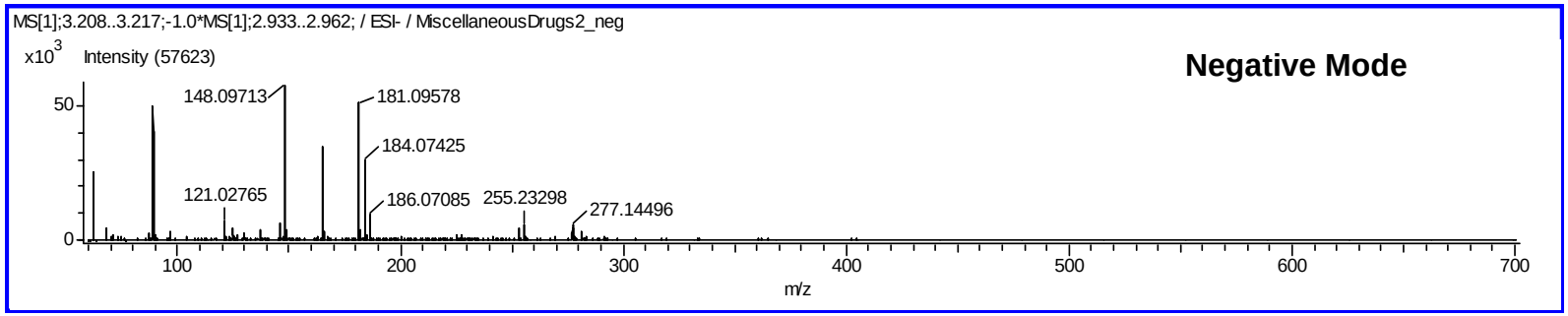




A-11. Miscellaneous Drugs (continued)

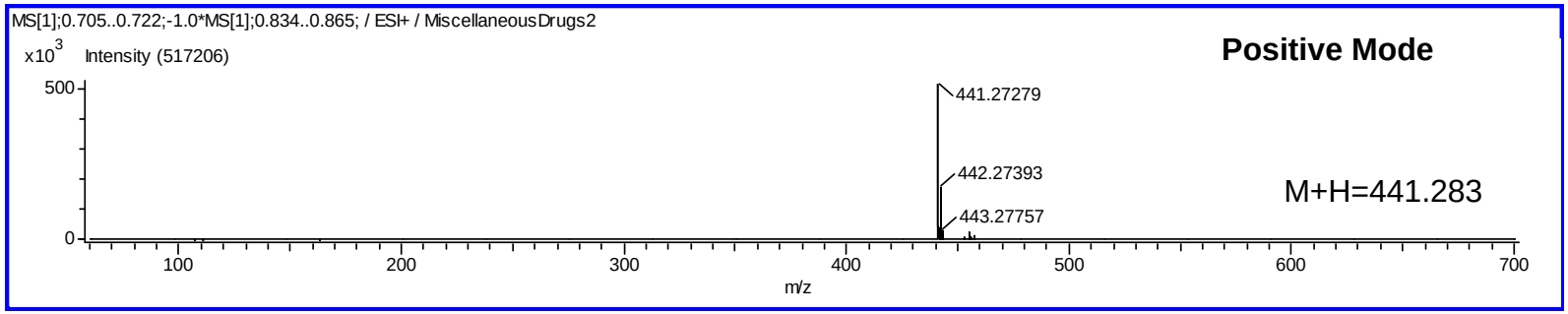
A-11.13. Triethanolamine

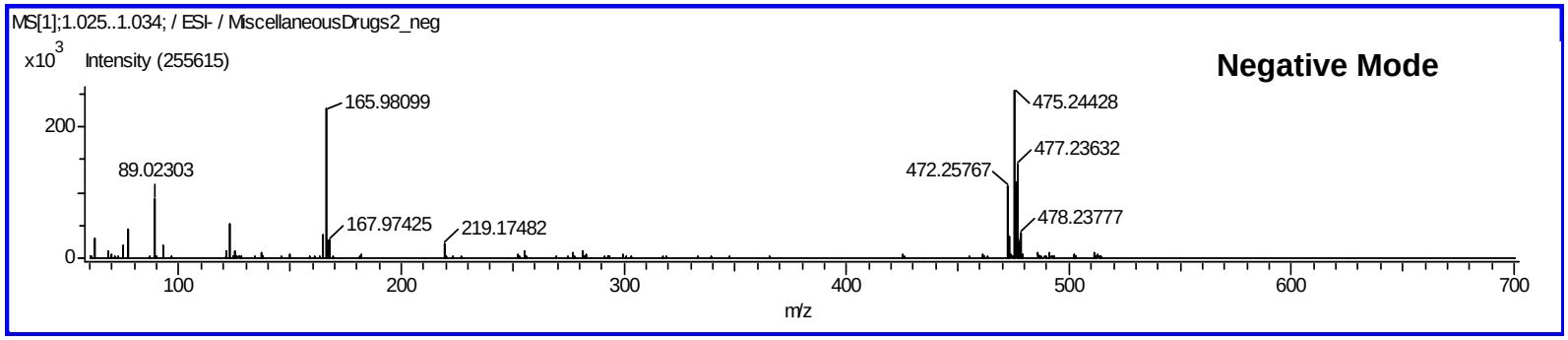




A-11. Miscellaneous Drugs (continued)

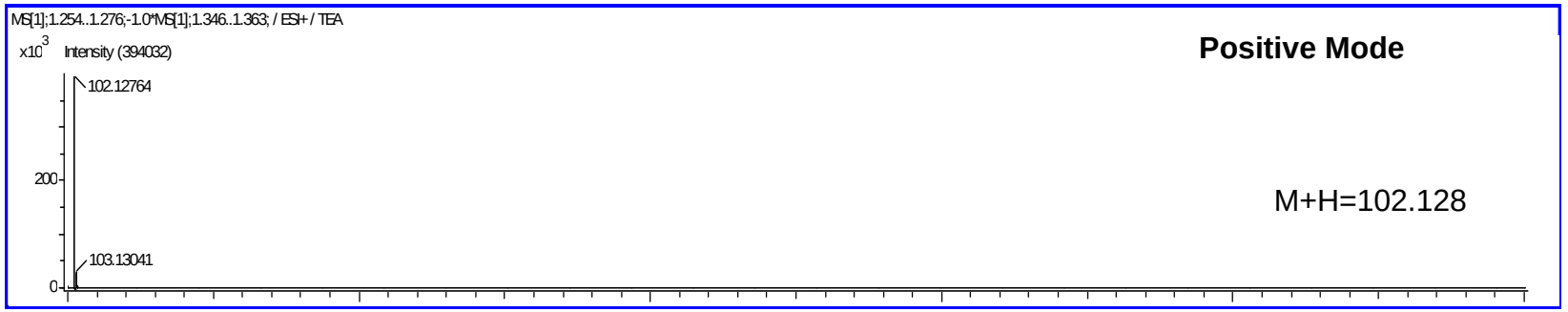
A-11.14. Norverapamil

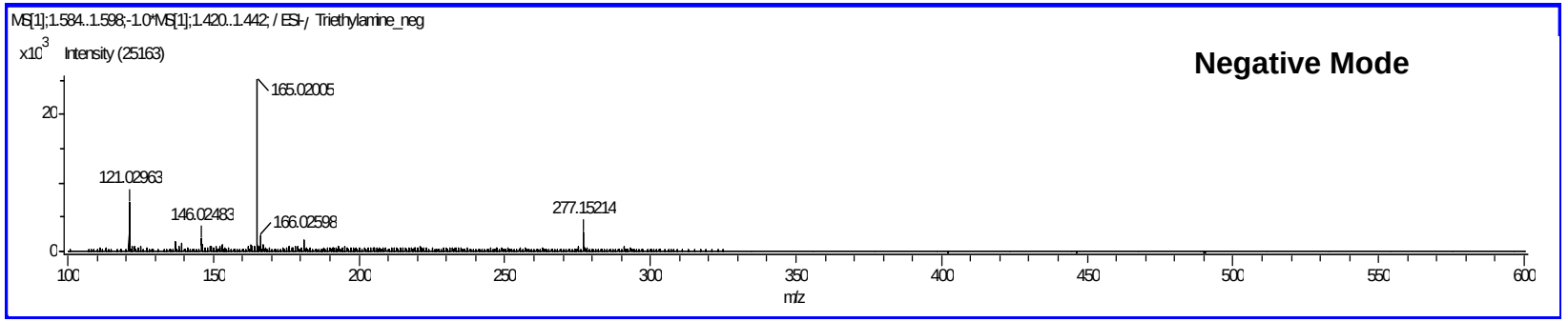




A-11. Miscellaneous Drugs (continued)

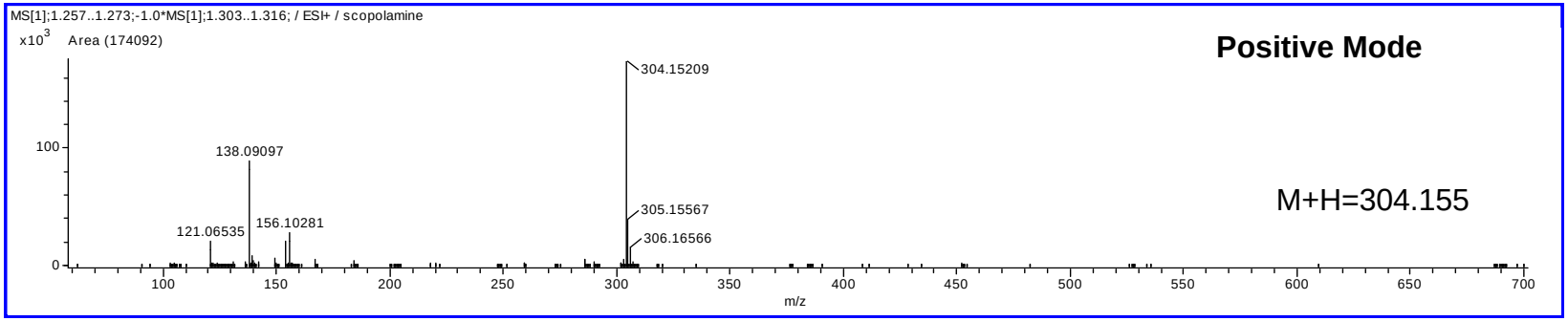
A-11.15. Triethylamine

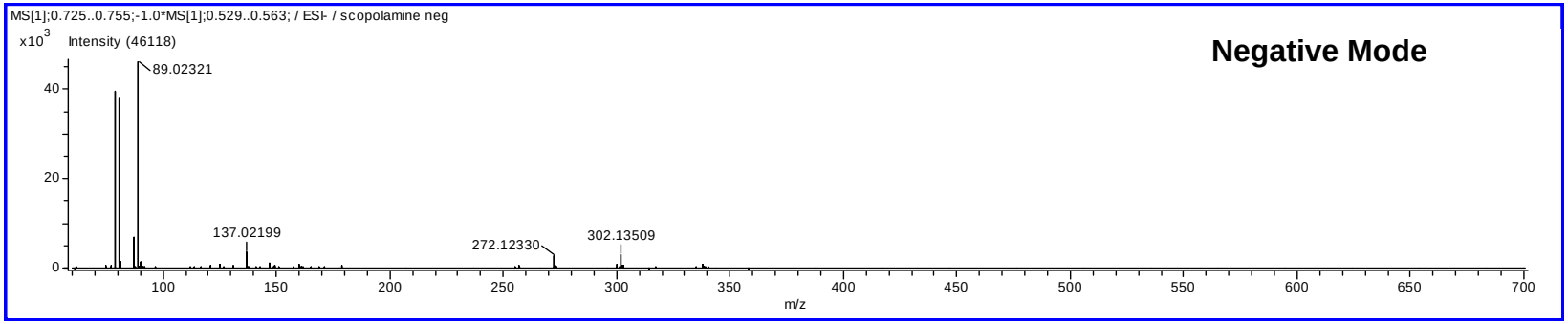




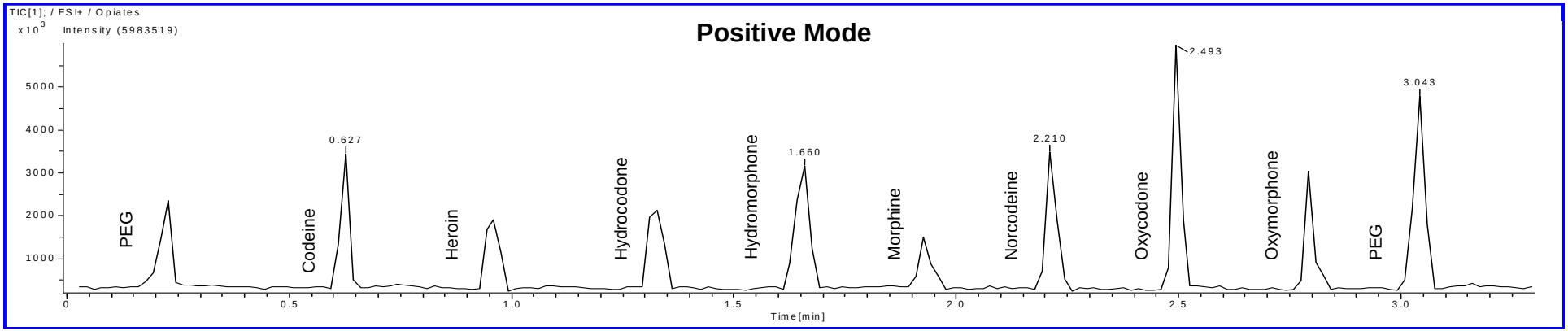
A-11. Miscellaneous Drugs (continued)

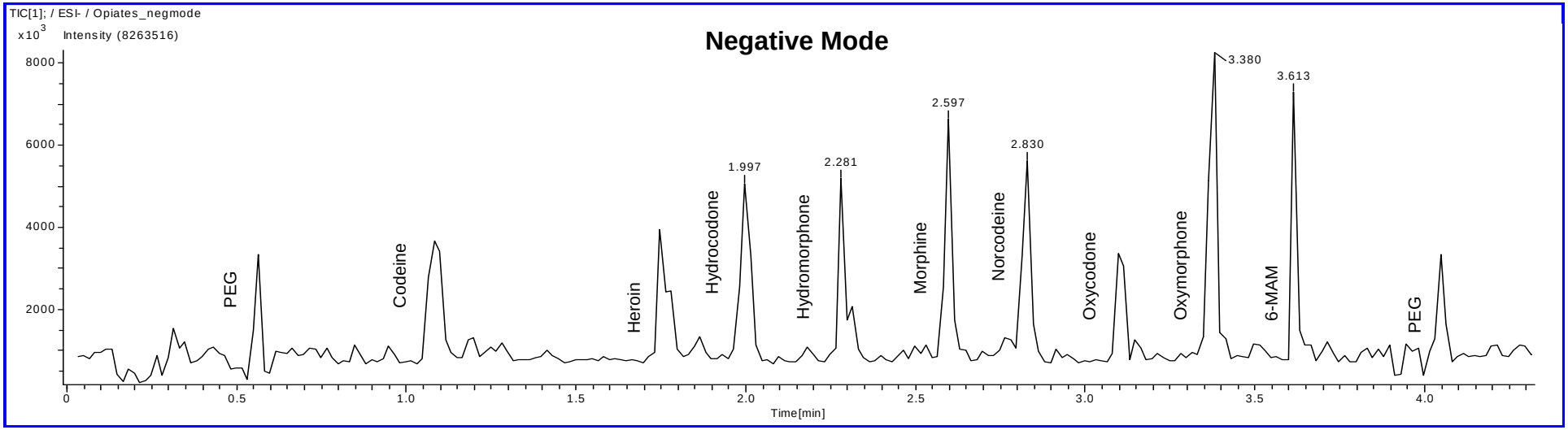
A-11.16. Scopolamine





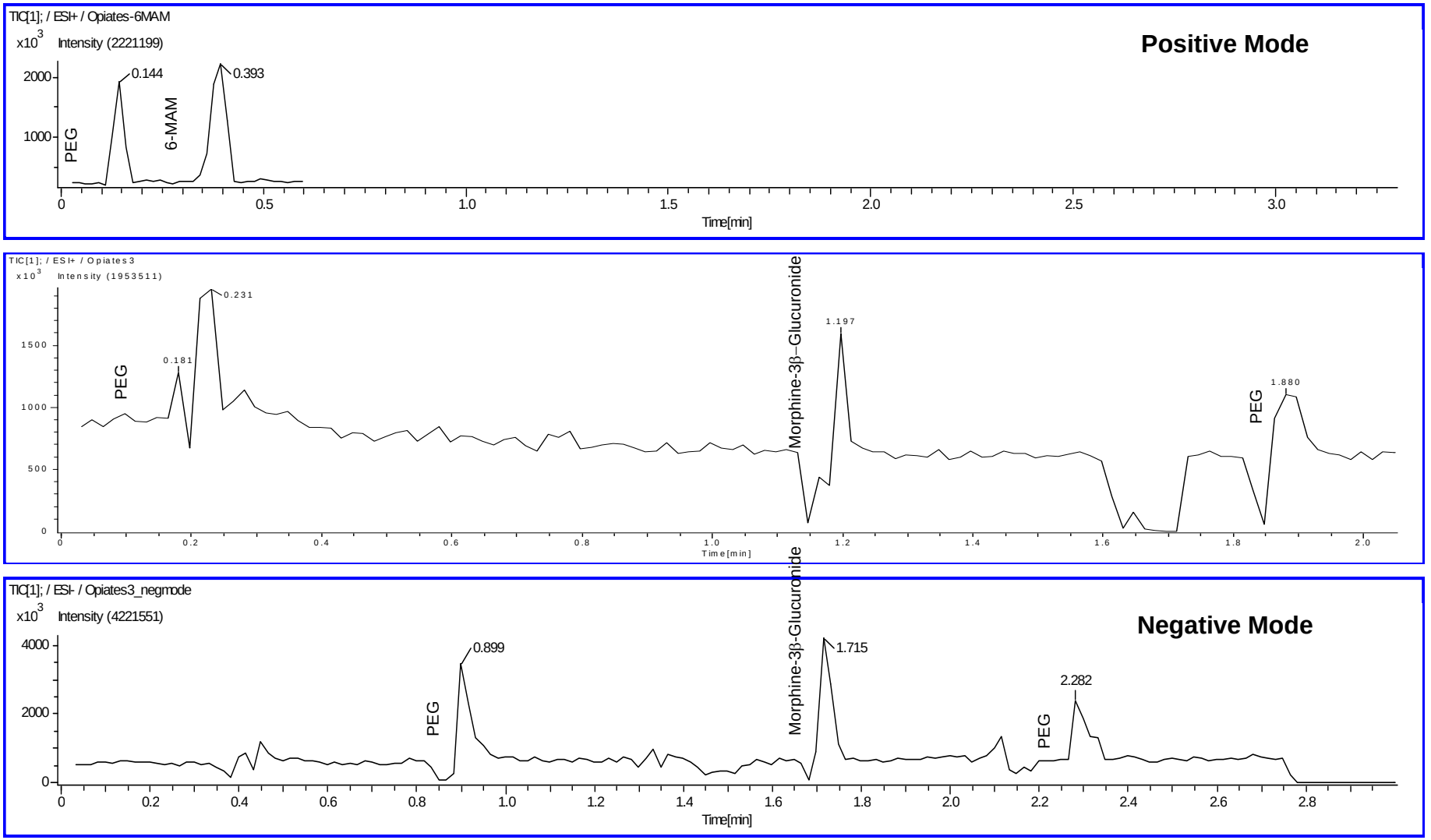
A-12. Opiates (4/11/07)





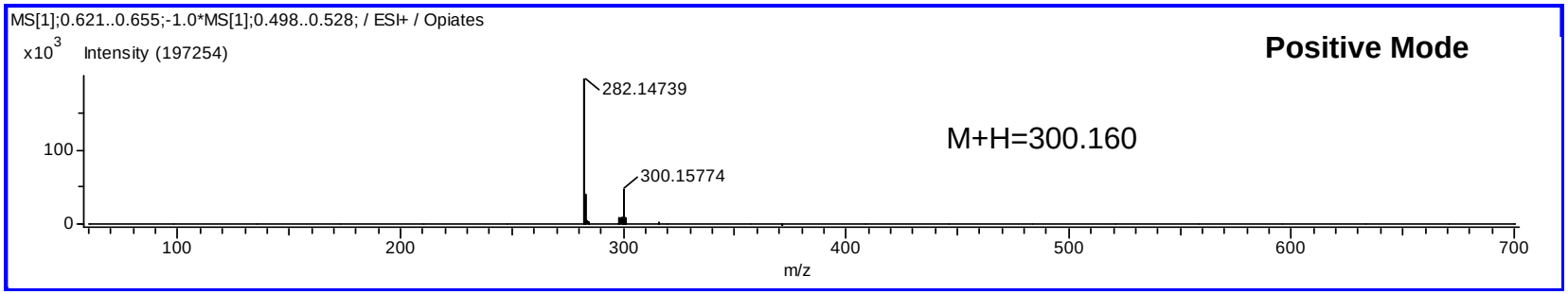
A-12. Opiates (continued)

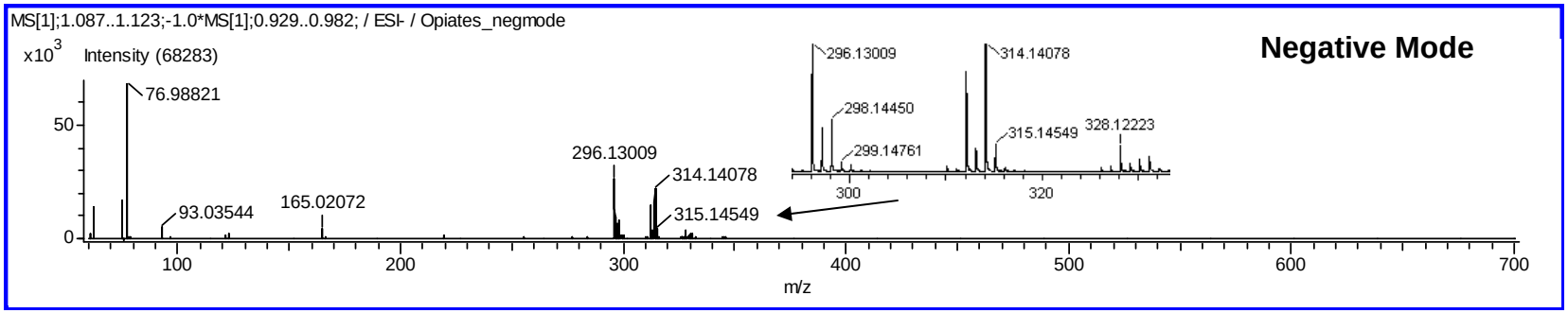
A-12.1. 6-MAM (4/11/07)



A-12. Opiates (continued)

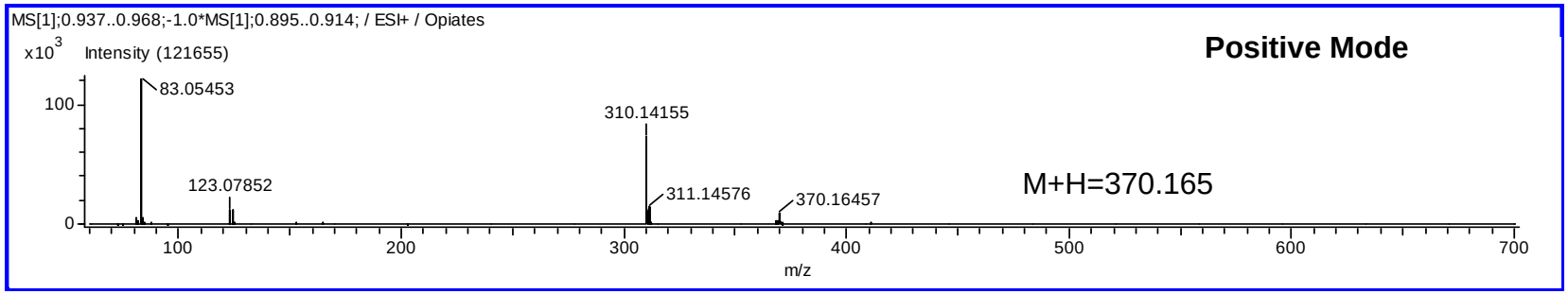
A-12.2. Codeine

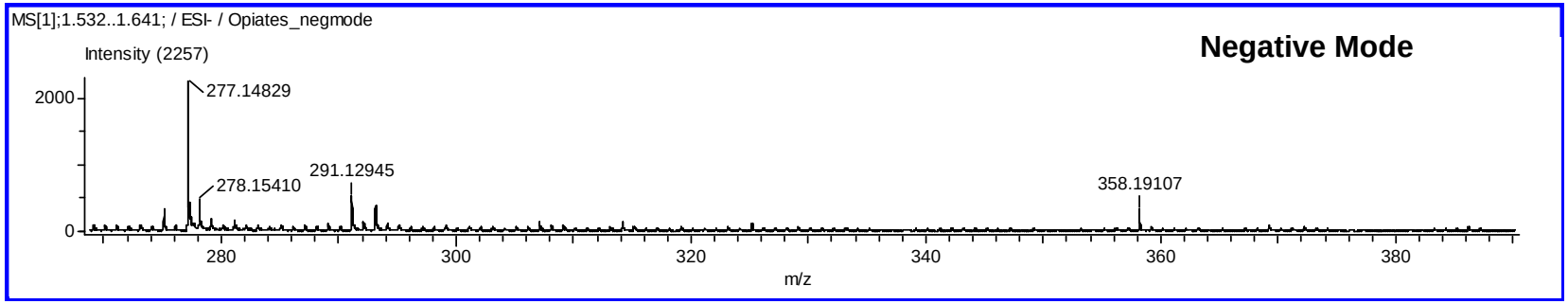




A-12. Opiates (continued)

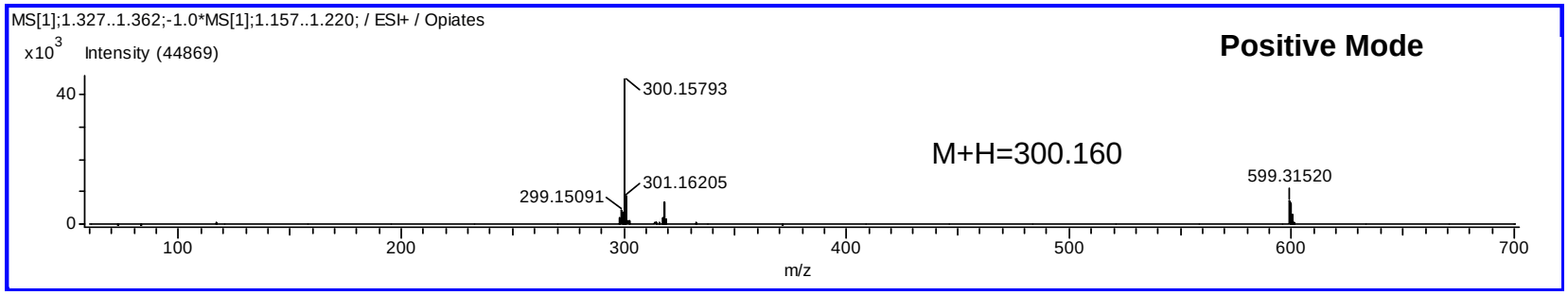
A-12.3. Heroin

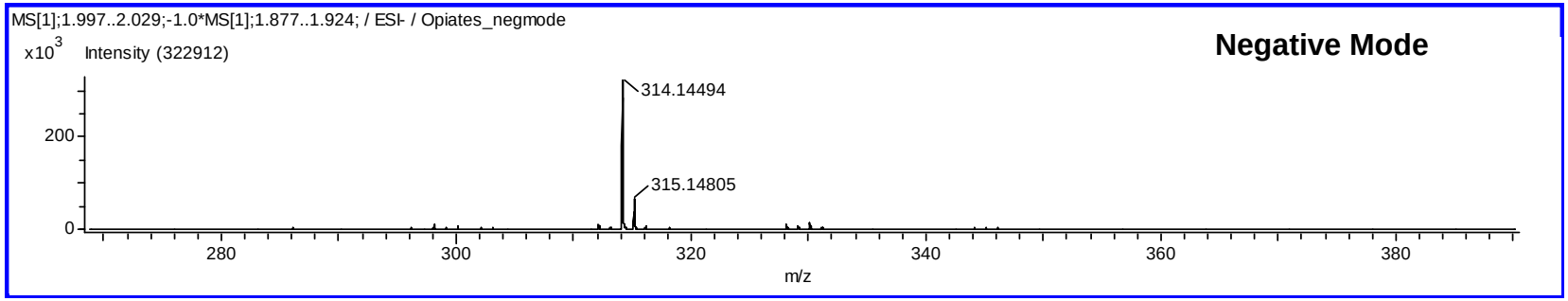




A-12. Opiates (continued)

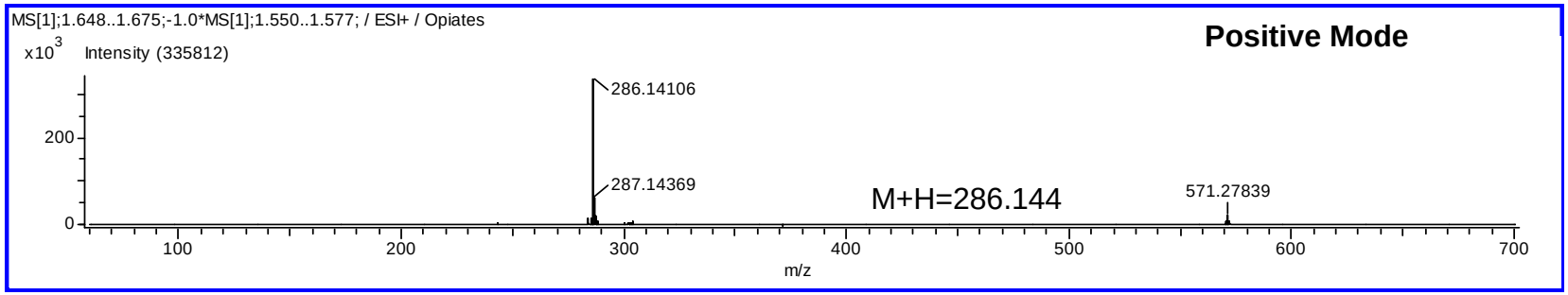
A-12.4. Hydrocodone

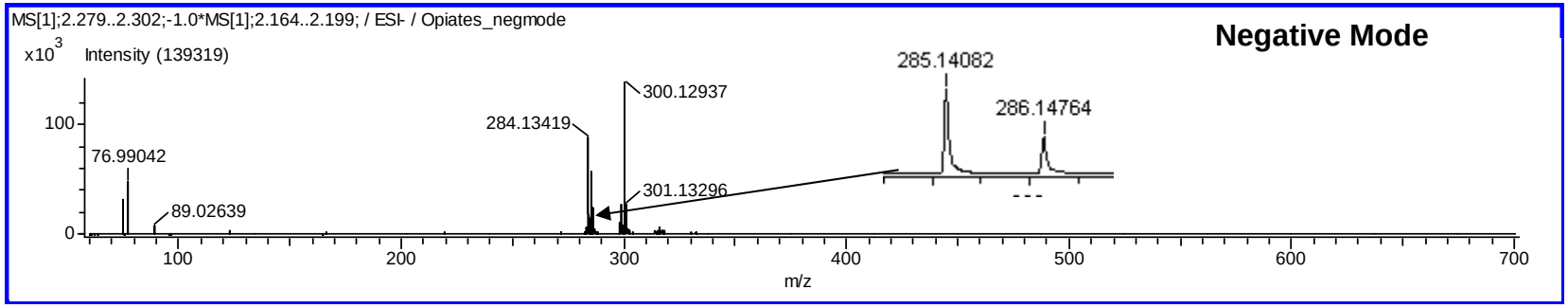




A-12. Opiates (continued)

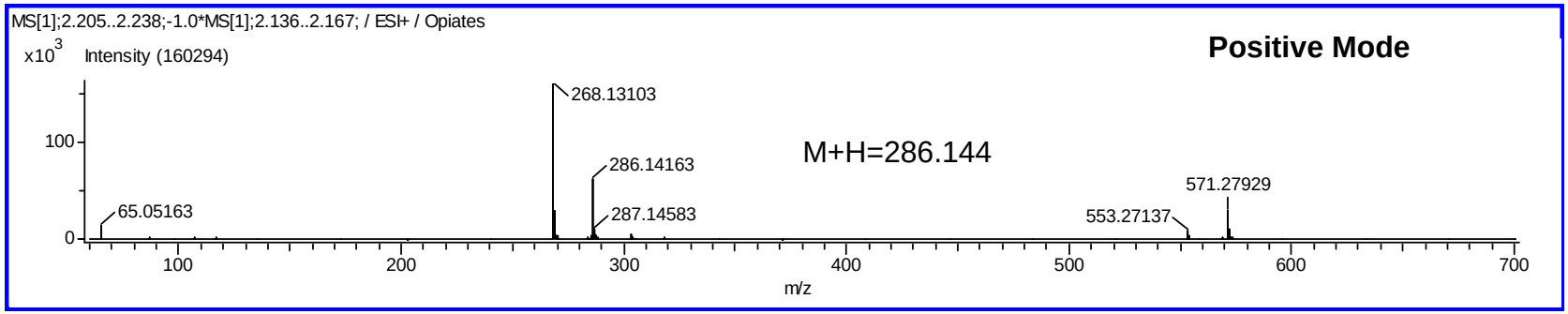
A-12.5. Hydromorphone

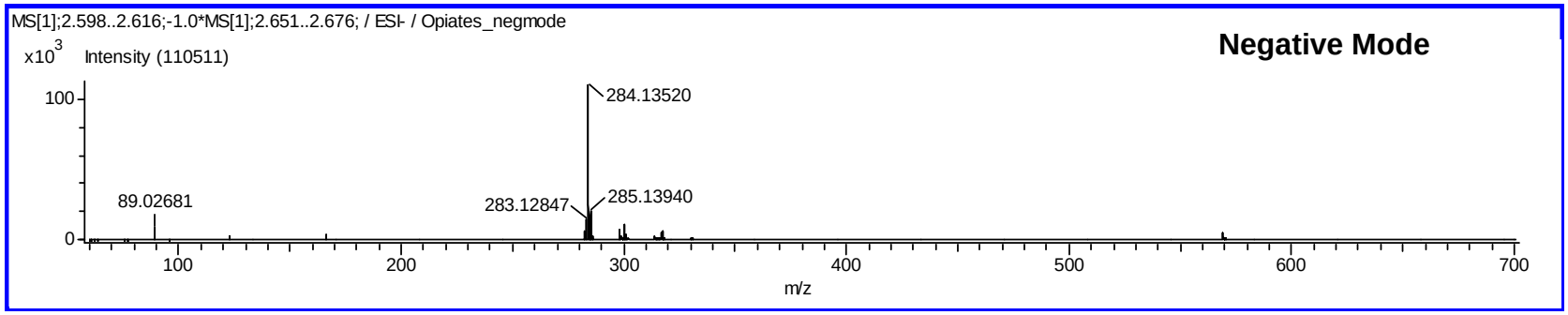




A-12. Opiates (continued)

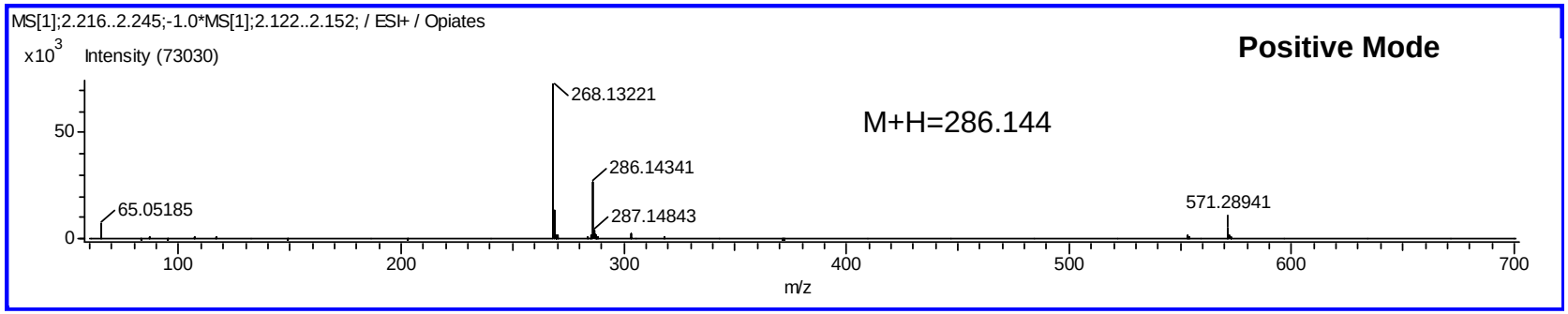
A-12.6. Morphine

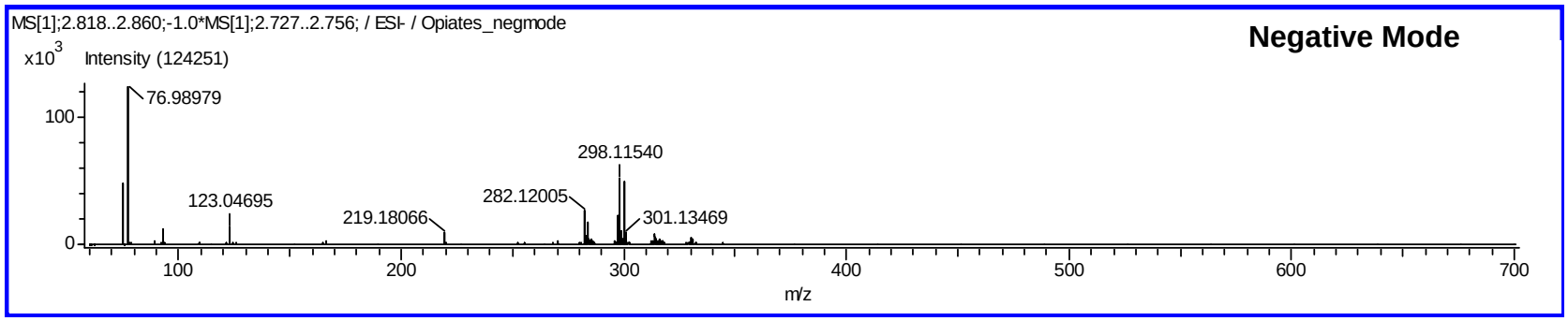




A-12. Opiates (continued)

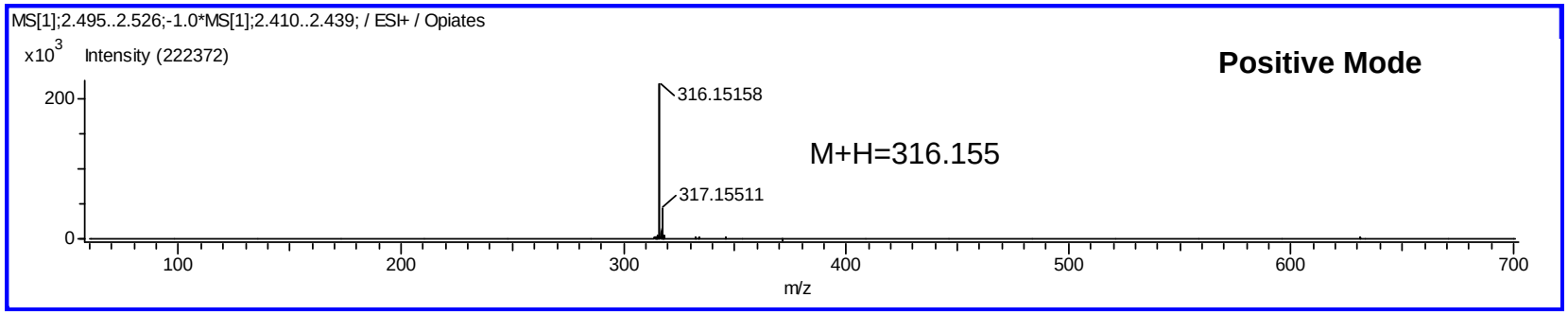
A-12.7. Norcodeine

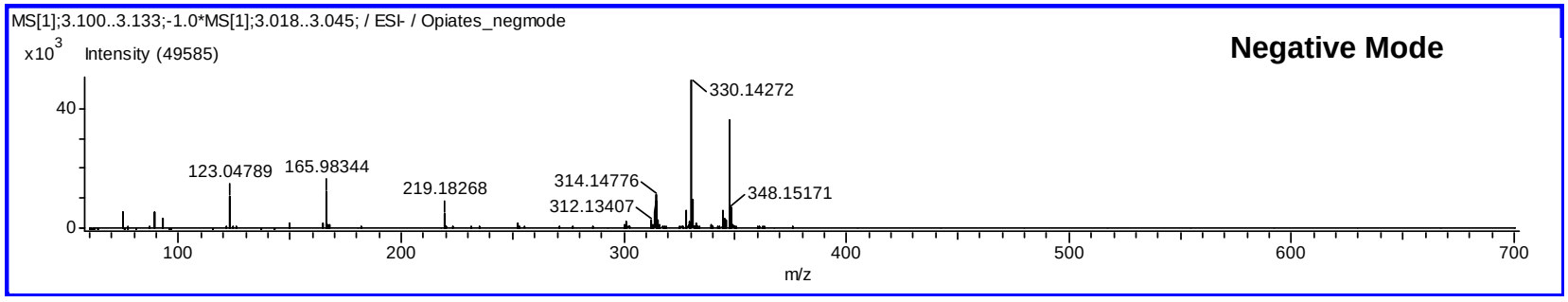




A-12. Opiates (continued)

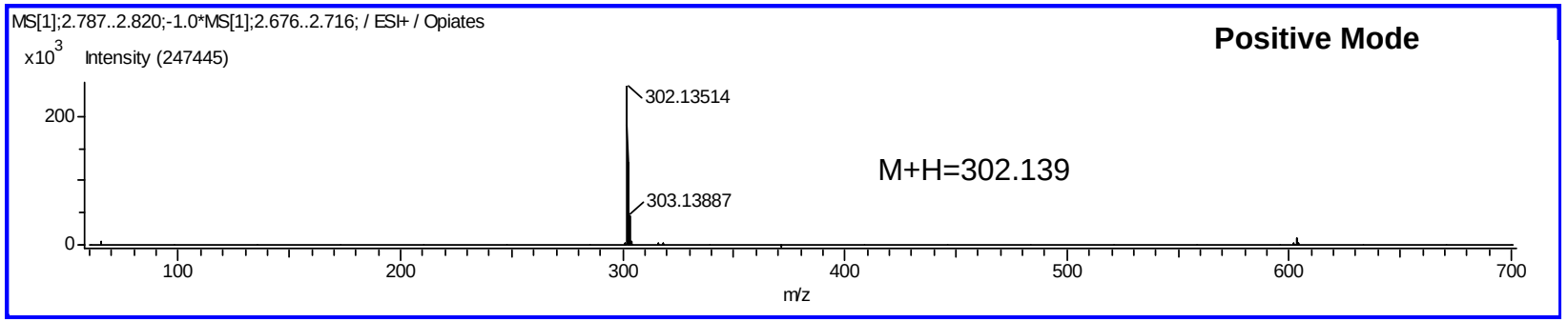
A-12.8. Oxycodone

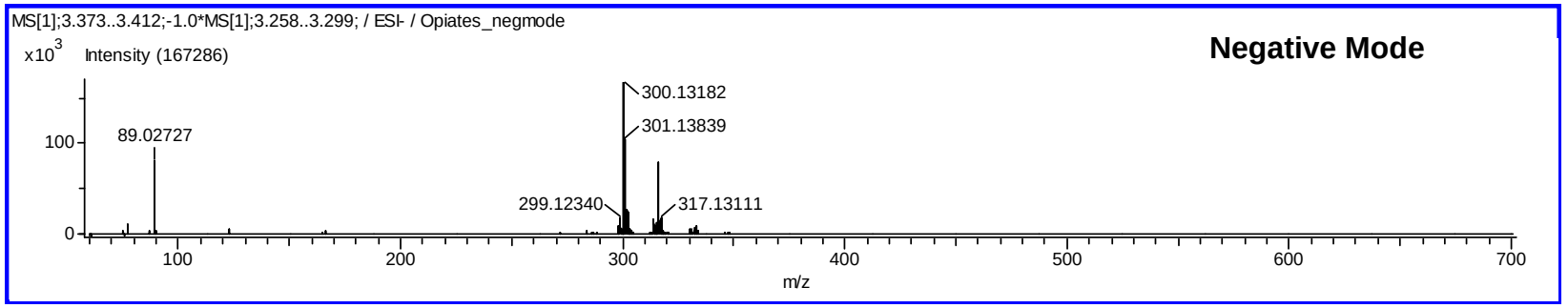




A-12. Opiates (continued)

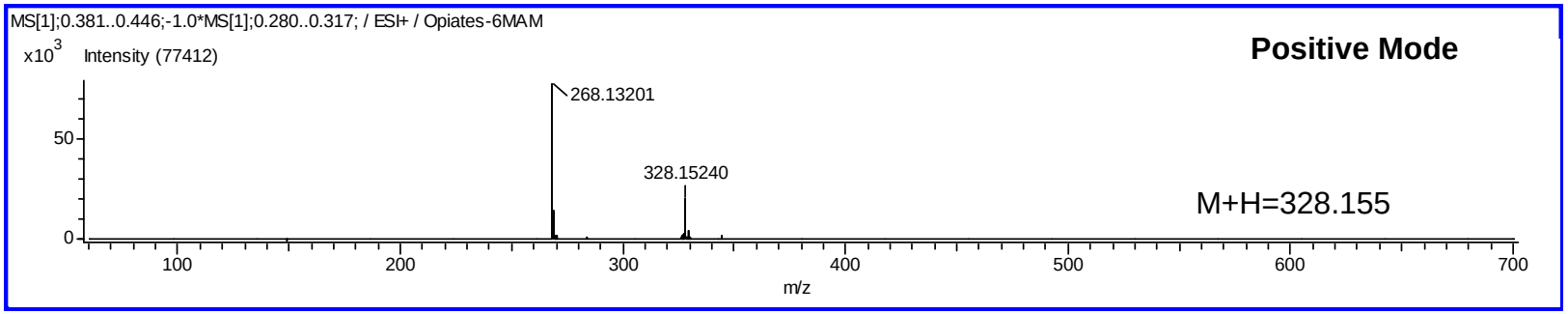
A-12.9. Oxymorphone

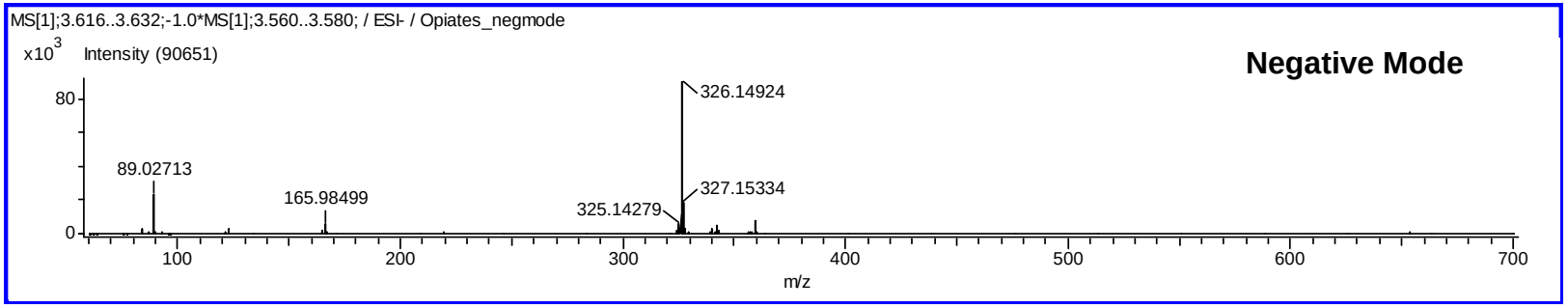




A-12. Opiates (continued)

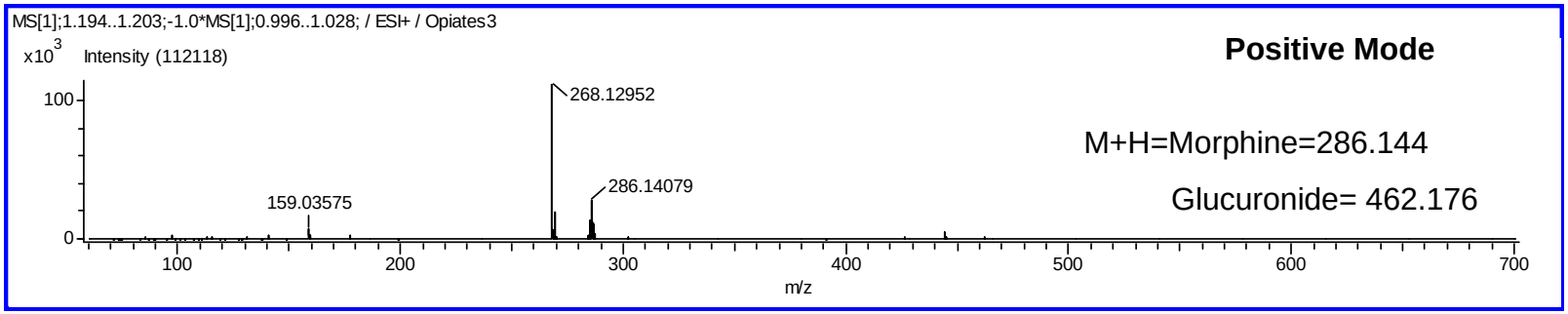
A-12.10. 6-MAM

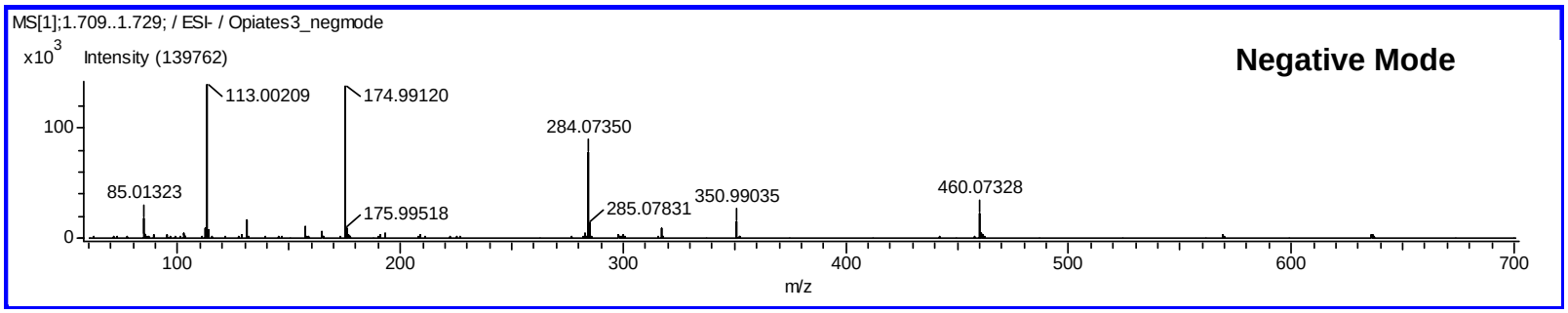




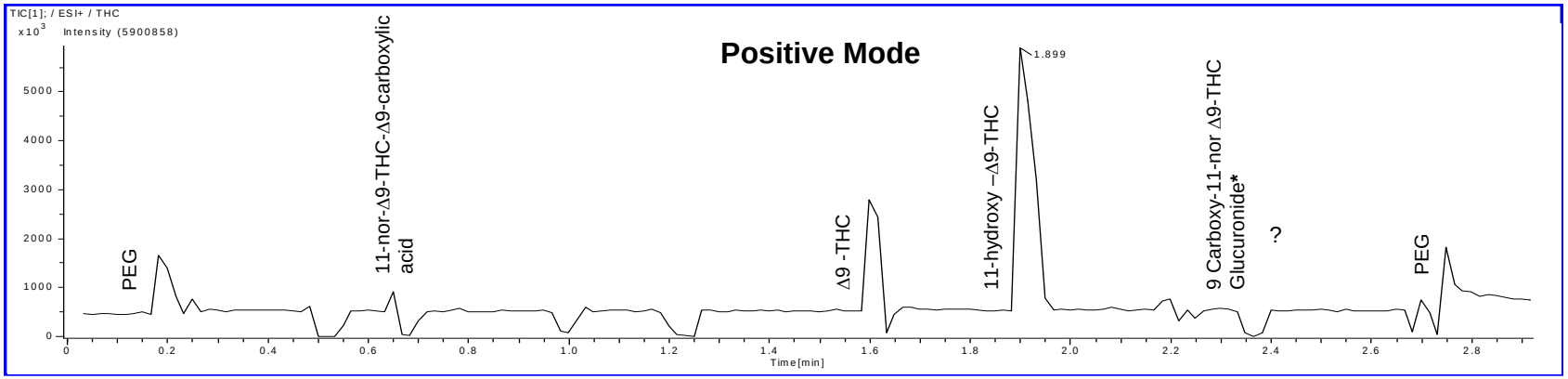
A-12. Opiates (continued)

A-12.11. Morphine-3β–Glucuronide

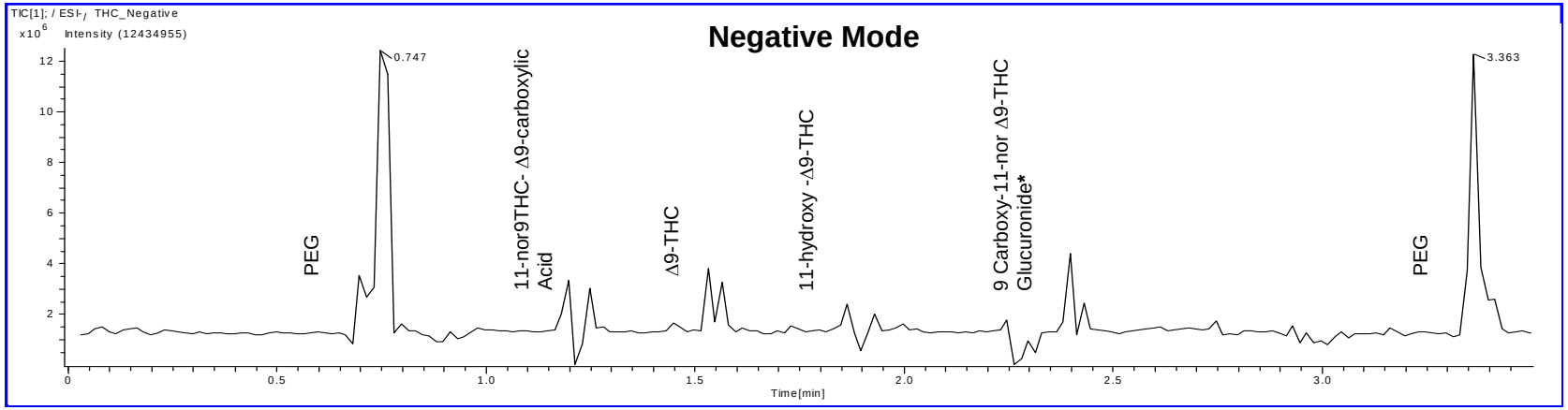




A-13. Cannabinoids (4/25/07)

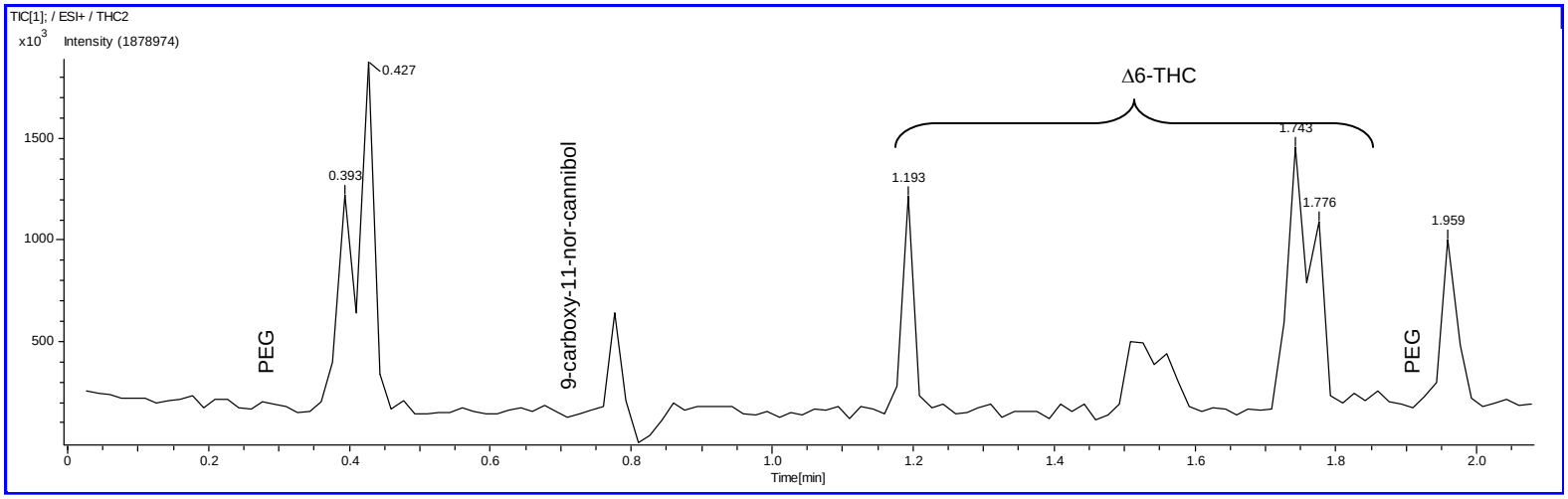


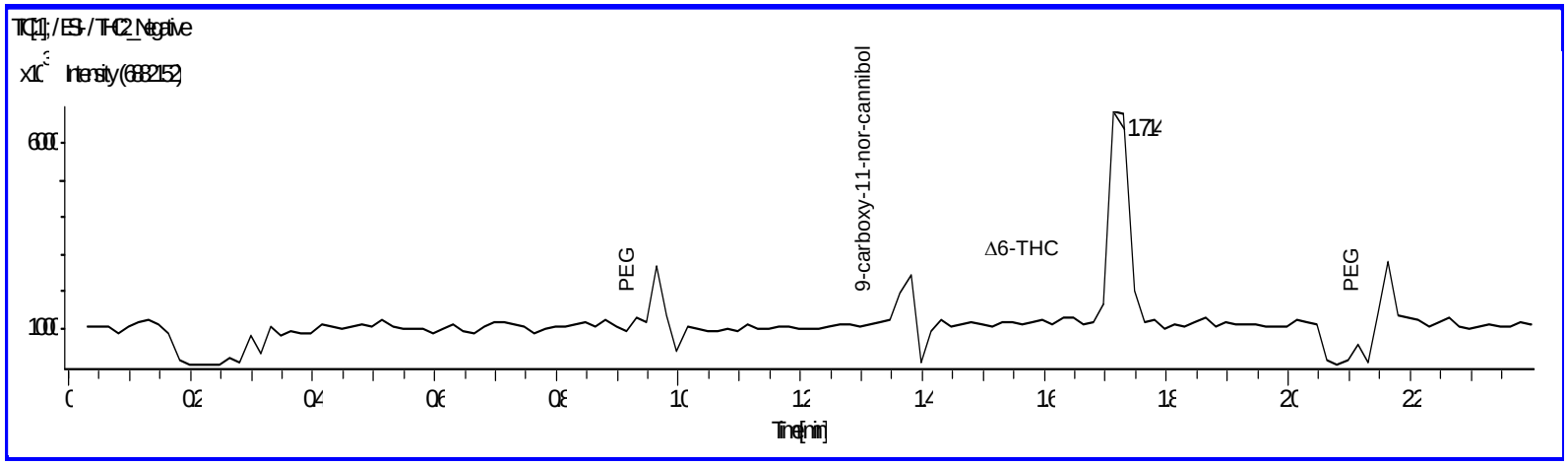
*Did not acquire spectrum in positive



A-13. Cannabinoids (continued)

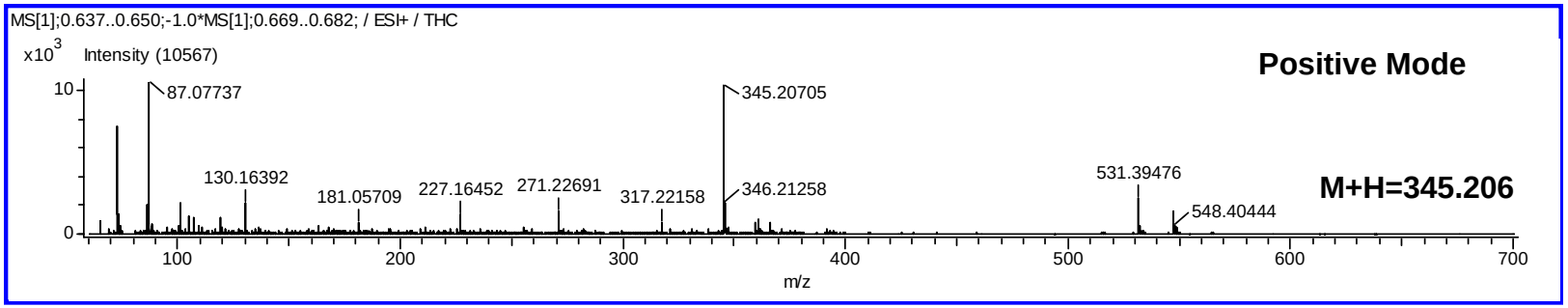
Positive Mode

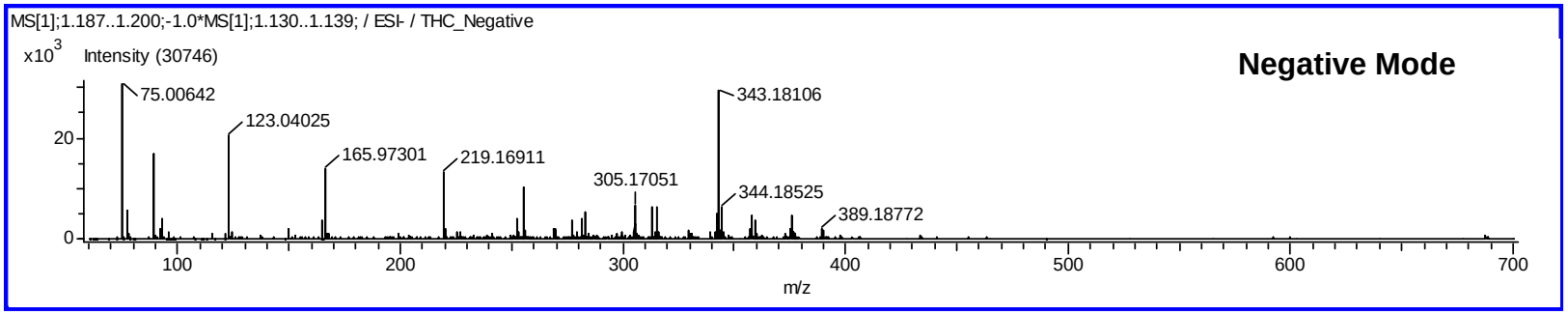




A-13. Cannabinoids (continued)

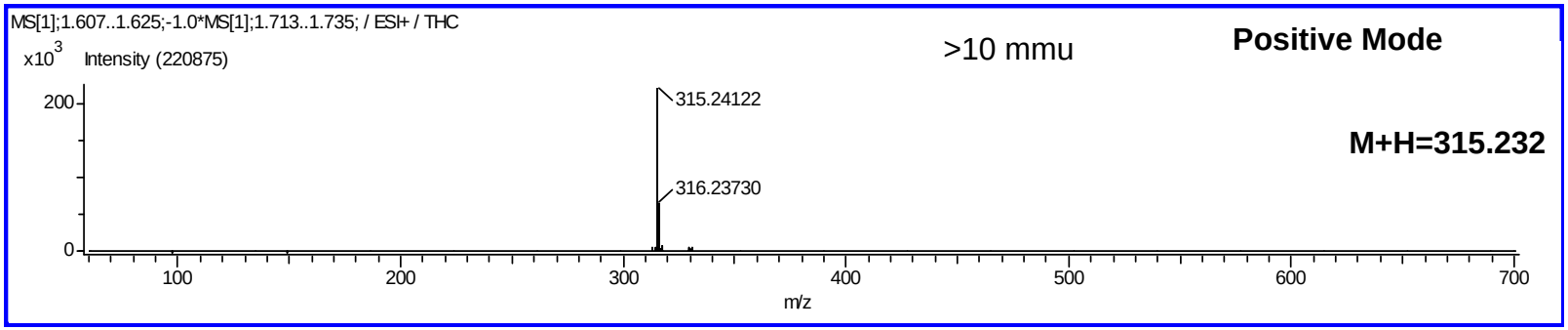
A-13.1. 11-nor9-THC-Δ9-carboxylic Acid

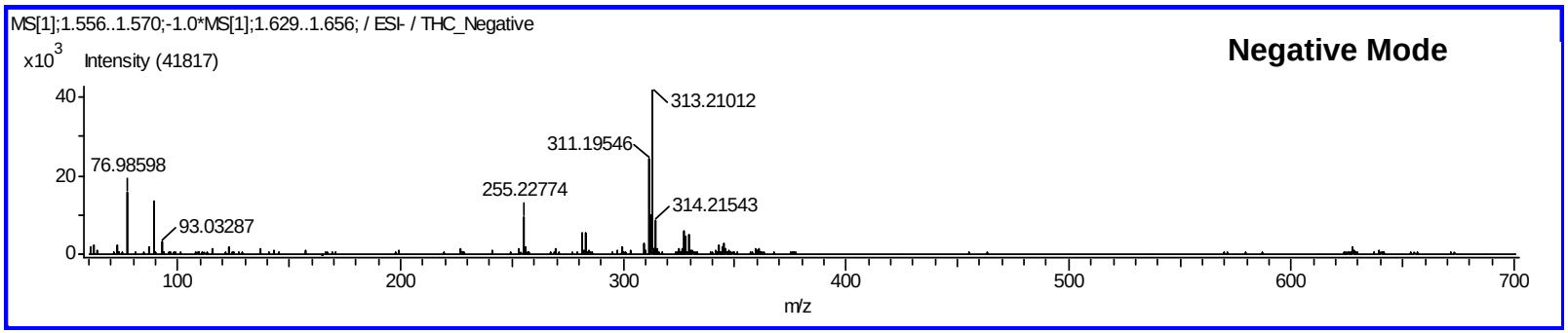




A-13. Cannabinoids (continued)

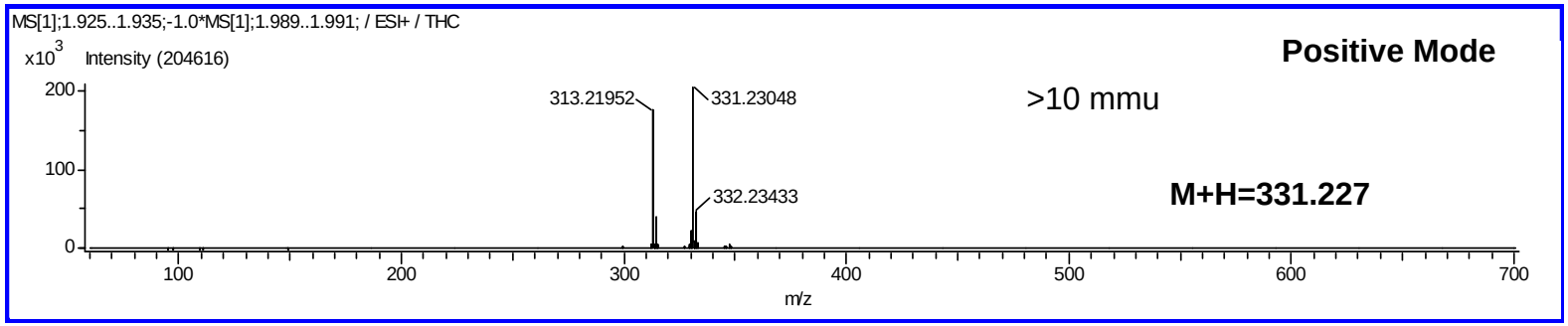
A-13.2. Δ9-THC

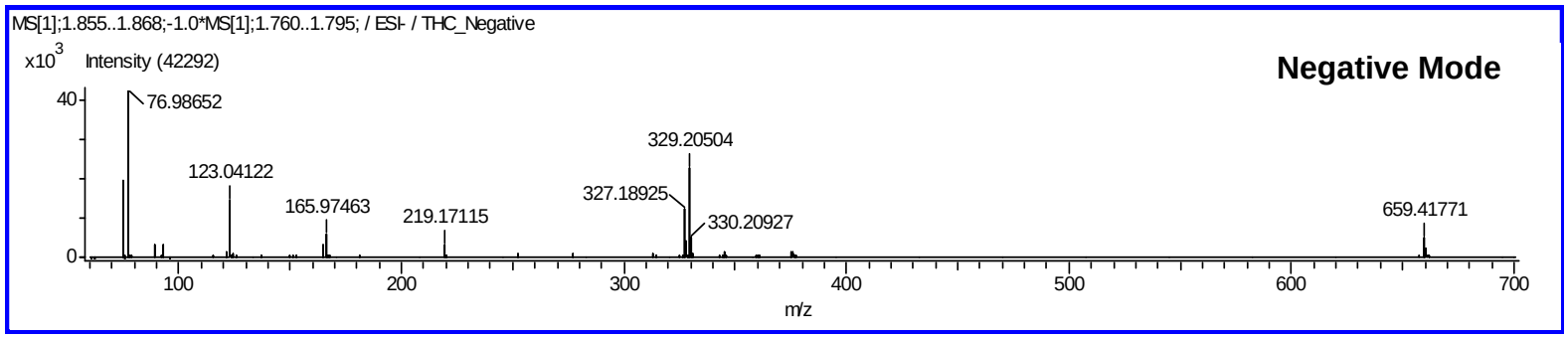




A-13. Cannabinoids (continued)

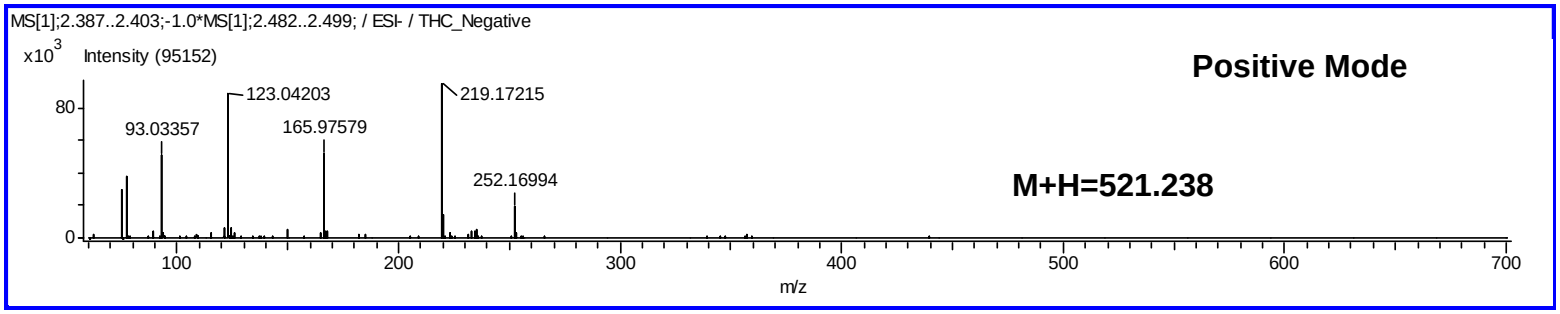
A-13.3. 11-hydroxy-Δ9-THC

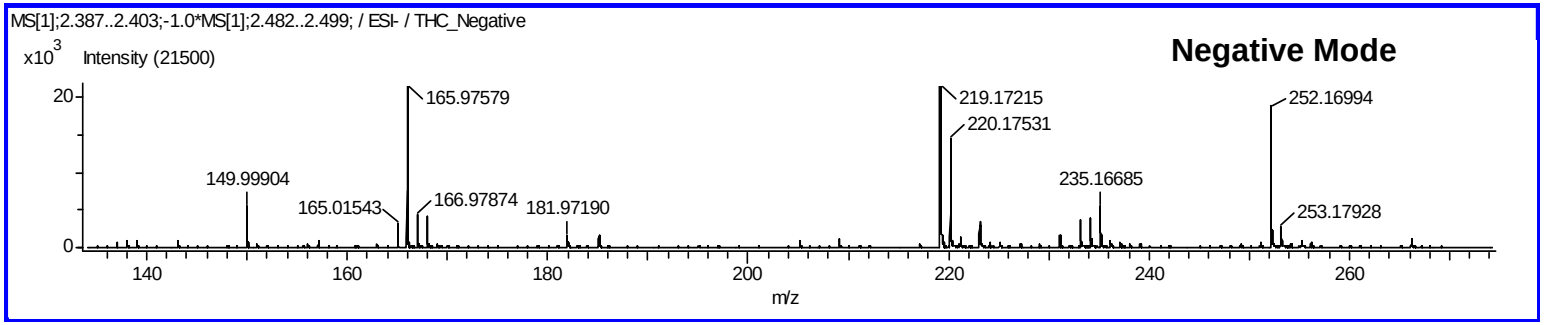




A-13. Cannabinoids (continued)

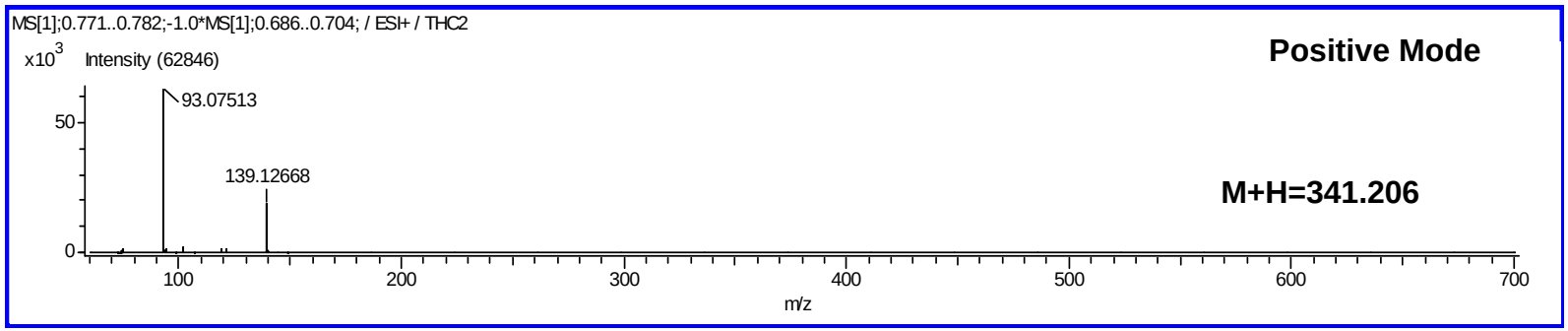
A-13.4. 9-carboxy-11-nor-Δ9-THC Glucuronide

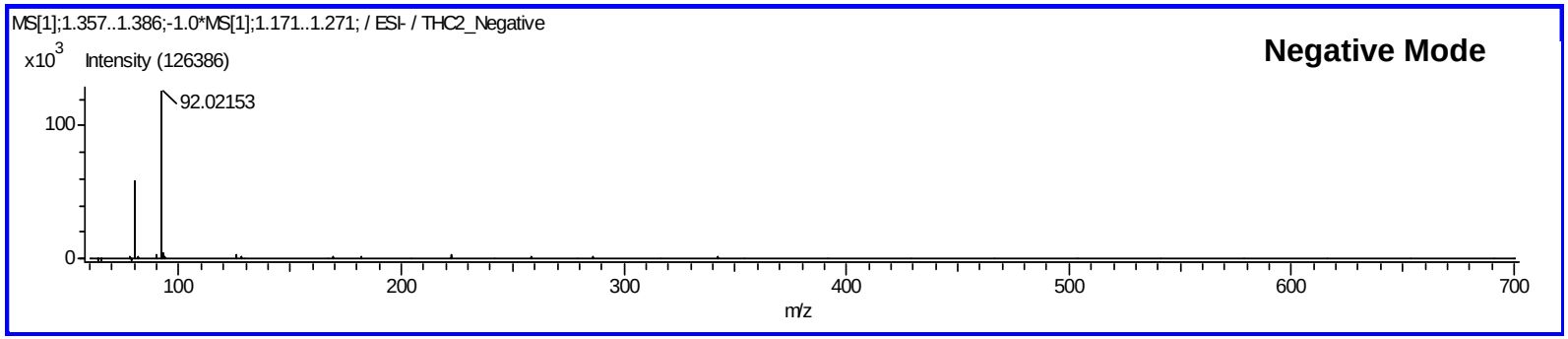




A-13. Cannabinoids (continued)

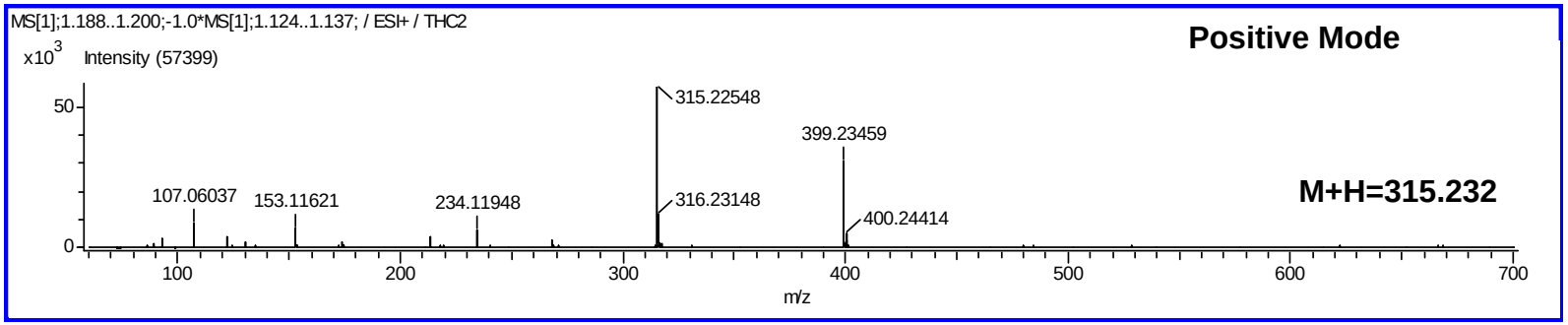
A-13.5. 9-carboxy-11-nor-cannibol

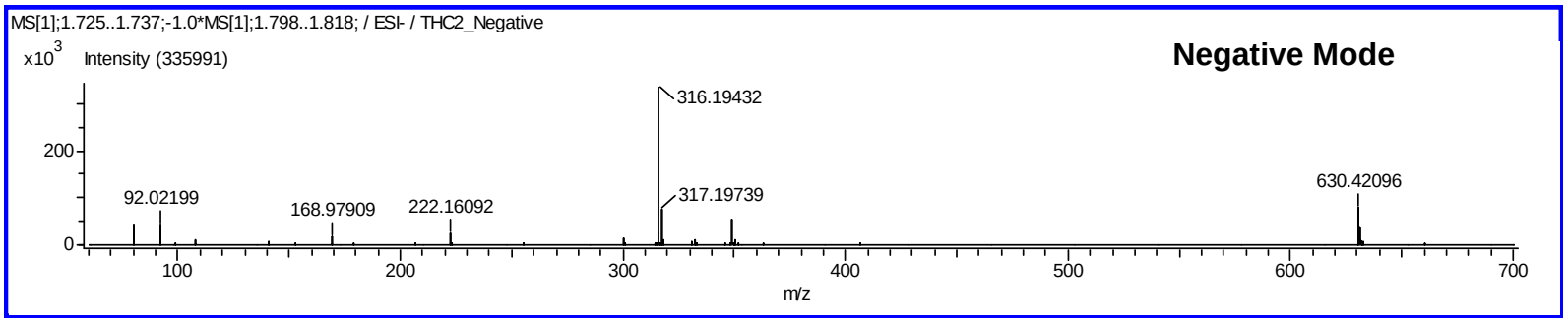




A-13. Cannabinoids (continued)

A-13.6. Δ6-THC





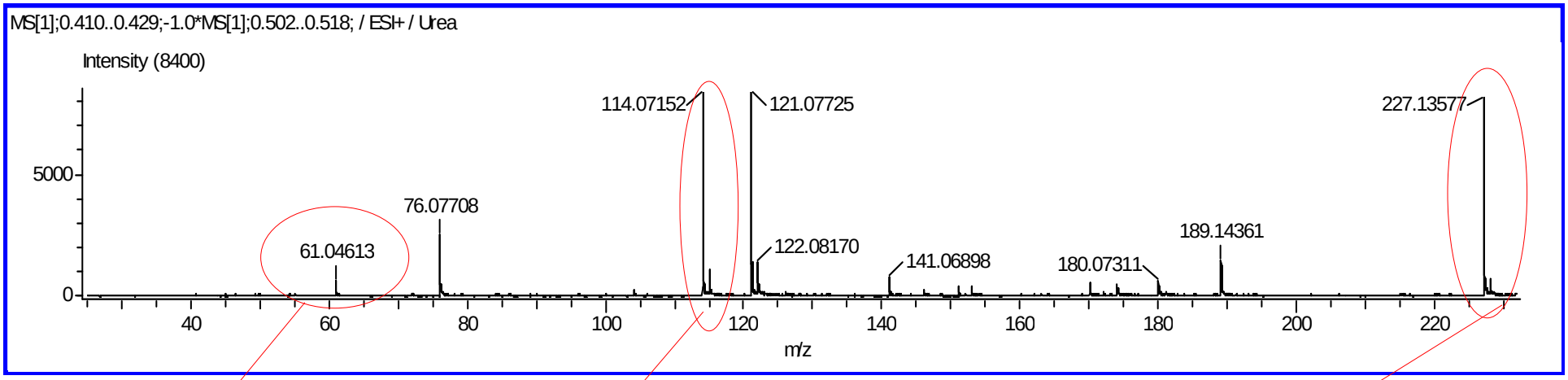
Appendix B

Drugs in Urine

Appendix B

Drugs in Urine

B-1. Urine Components



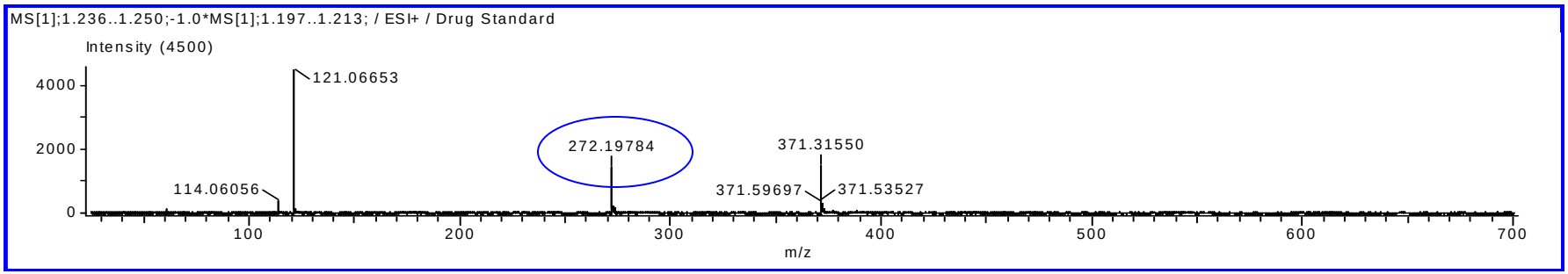
Urea+H

Creatinine+H

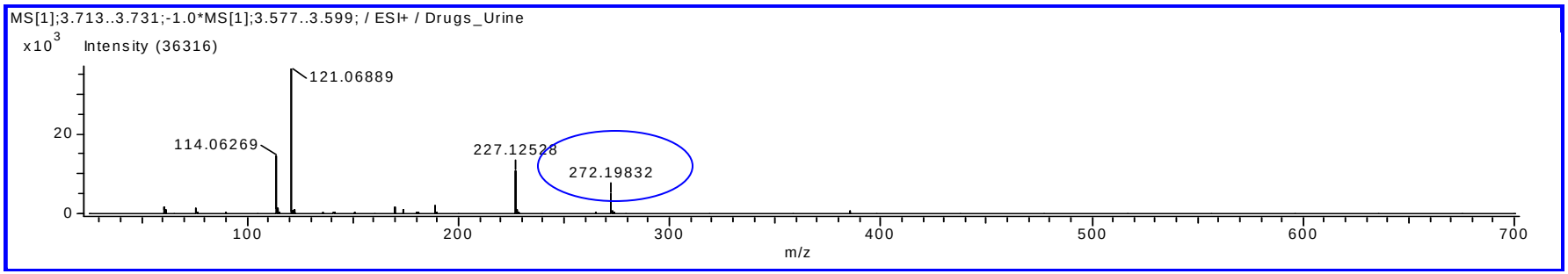
Creatinine Dimer +H

B-2. Allergy/Cold

B-2.1. Dextromethorphan 100 μg/mL 272.200

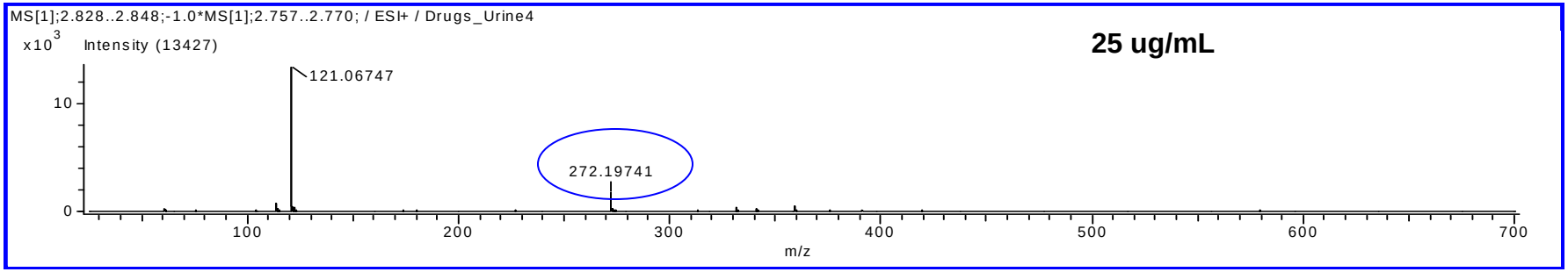


B-2.2. Dextromethorphan 50 μg/mL 272.200

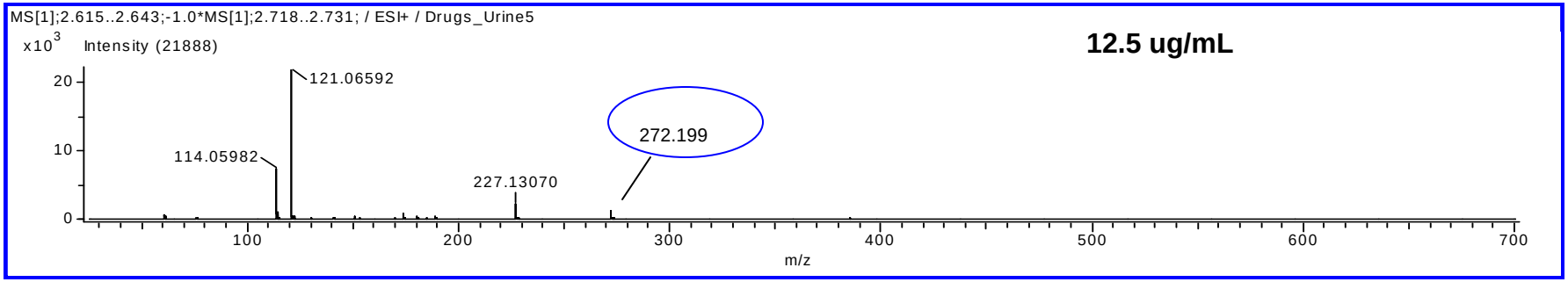


B-2. Allergy/Cold (continued)

B-2.3. Dextromethorphan 25 μg/mL 272.200

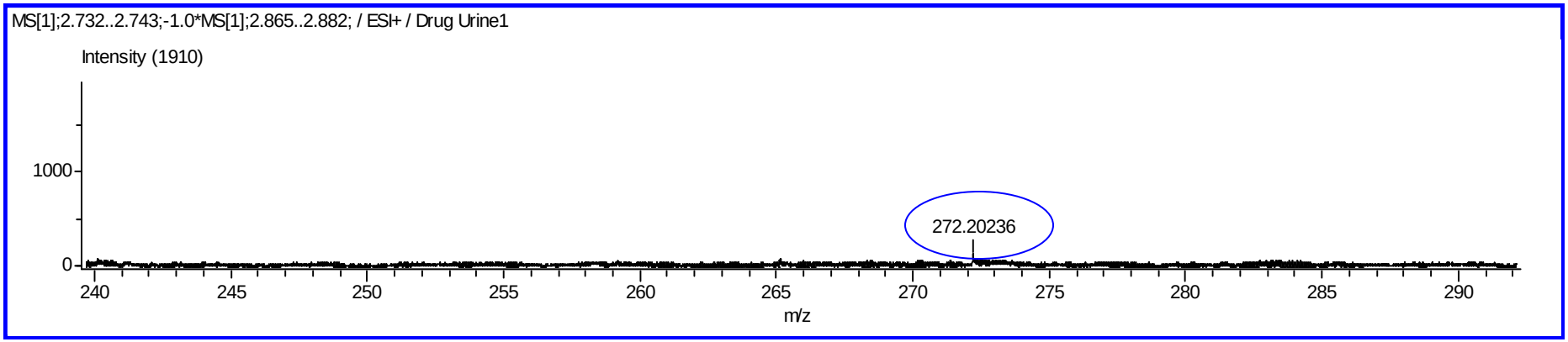


B-2.4. Dextromethorphan 12.5 μg/mL 272.200

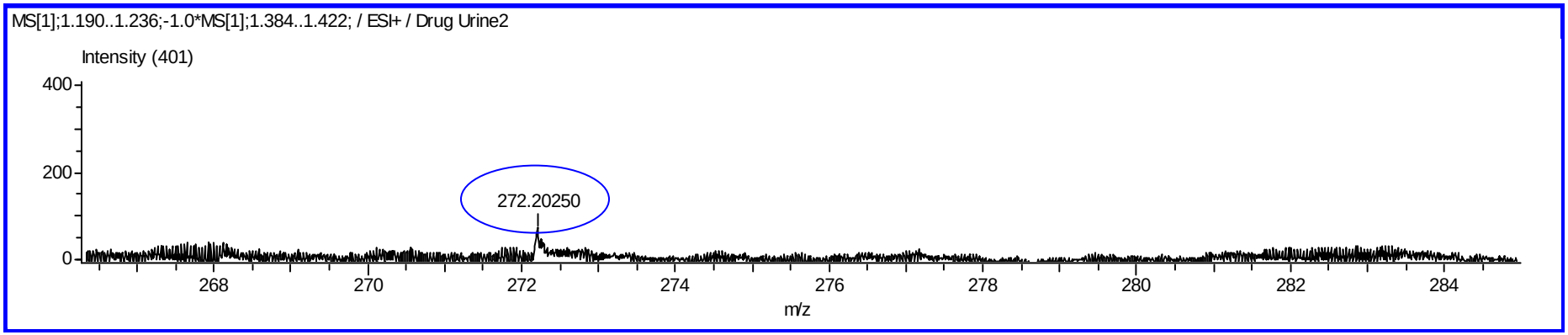


B-2. Allergy/Cold (continued)

B-2.5. Dextromethorphan 6.25 μg/mL 272.200

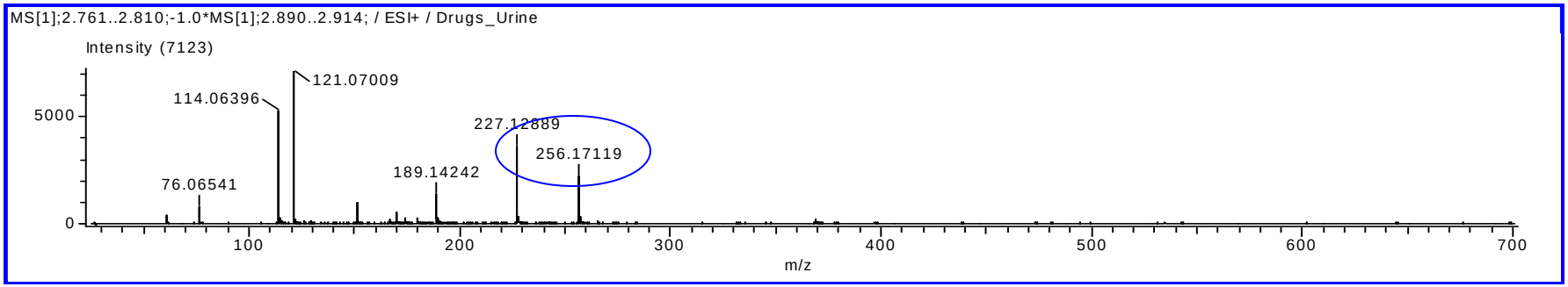


B-2.6. Dextromethorphan 3.125 μg/mL 272.200

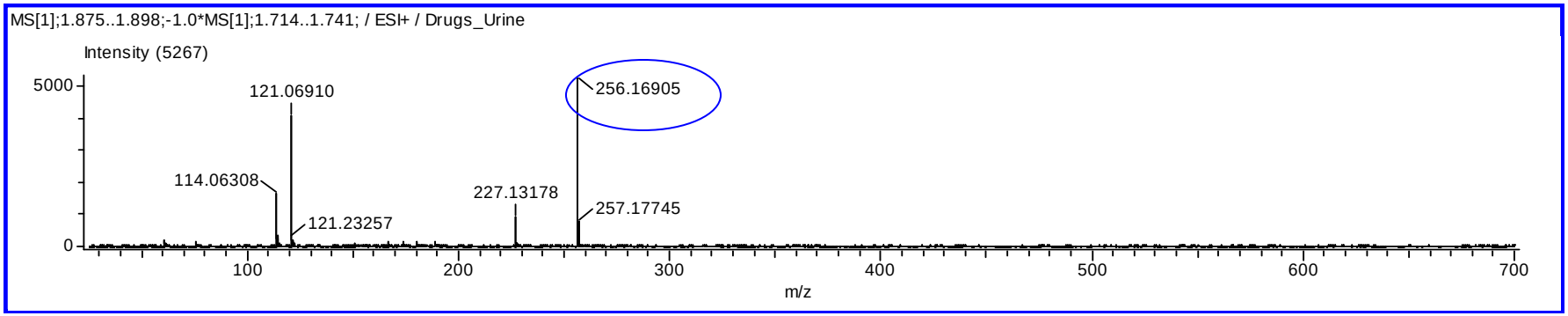


B-2. Allergy/Cold (continued)

B-2.7. Diphenhydramine 100 μg/mL 256.169

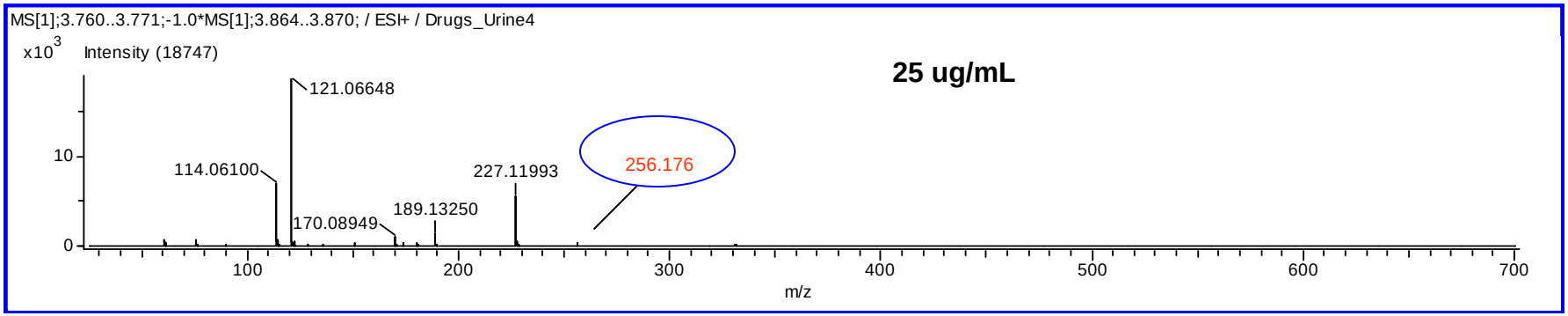


B-2.8. Diphenhydramine 50 μg/mL 256.169

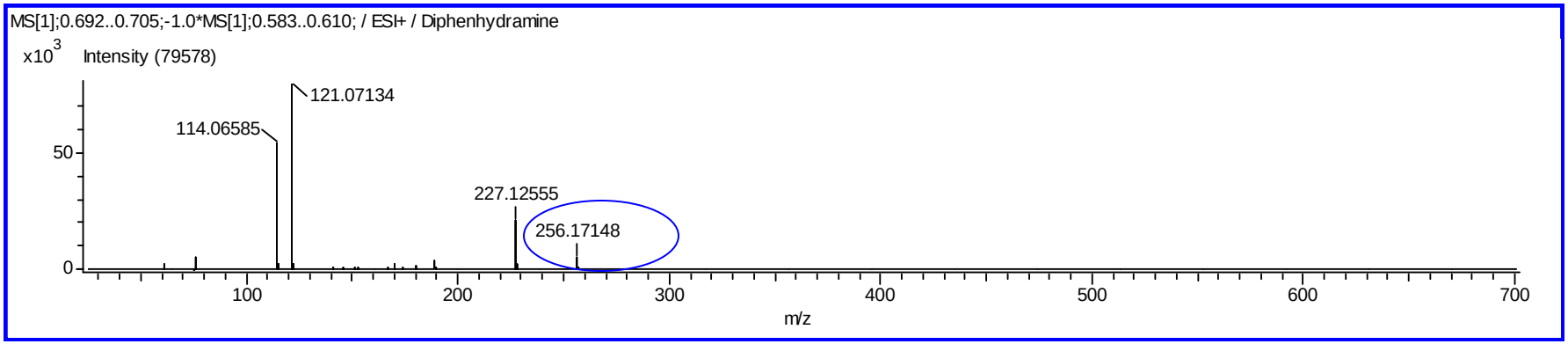


B-2. Allergy/Cold (continued)

B-2.9. Diphenhydramine 25 μg/mL 256.169

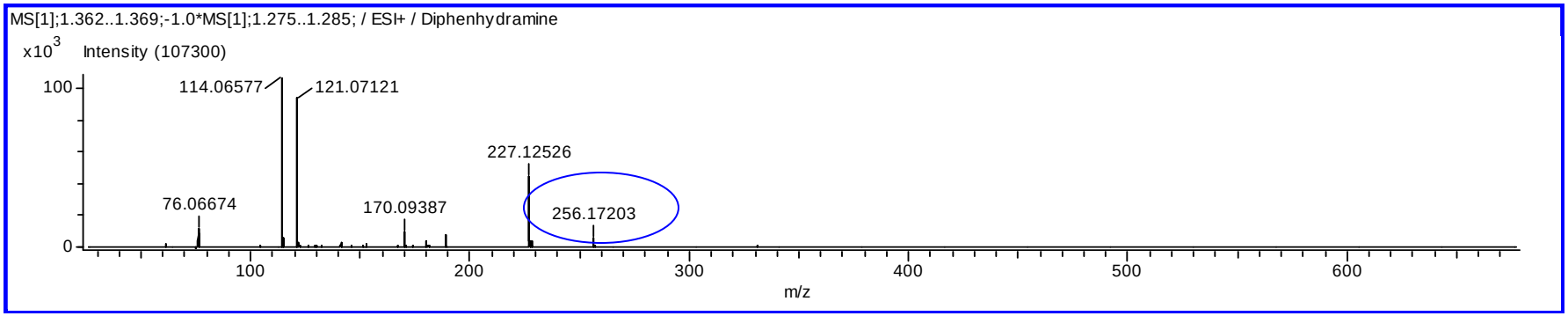


B-2.10. Diphenhydramine 12.5 μg/mL 256.169

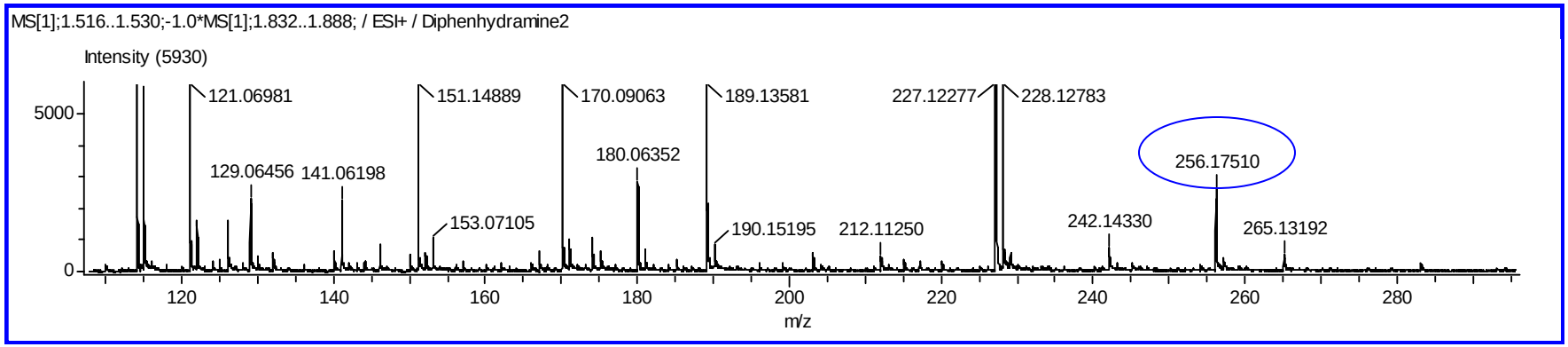


B-2. Allergy/Cold (continued)

B-2.11. Diphenhydramine 6.25 μg/mL 256.169

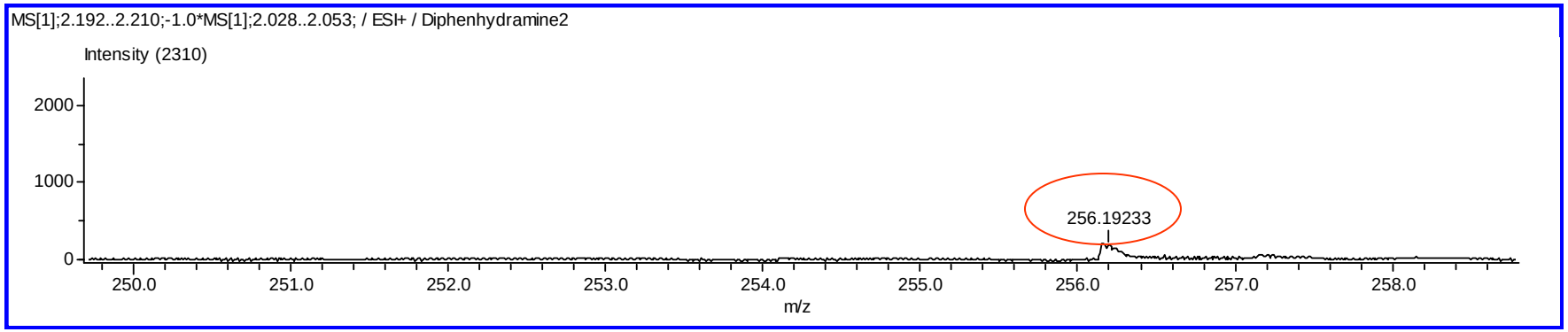


B-2.12. Diphenhydramine 3.125 μg/mL 256.169



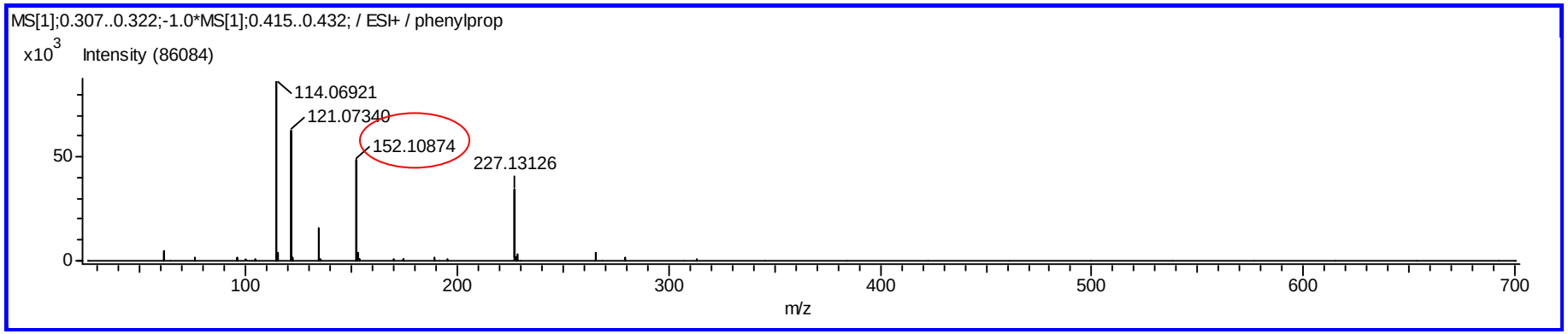
B-2. Allergy/Cold (continued)

B-2.13. Diphenhydramine 1.56 μg/mL 256.169

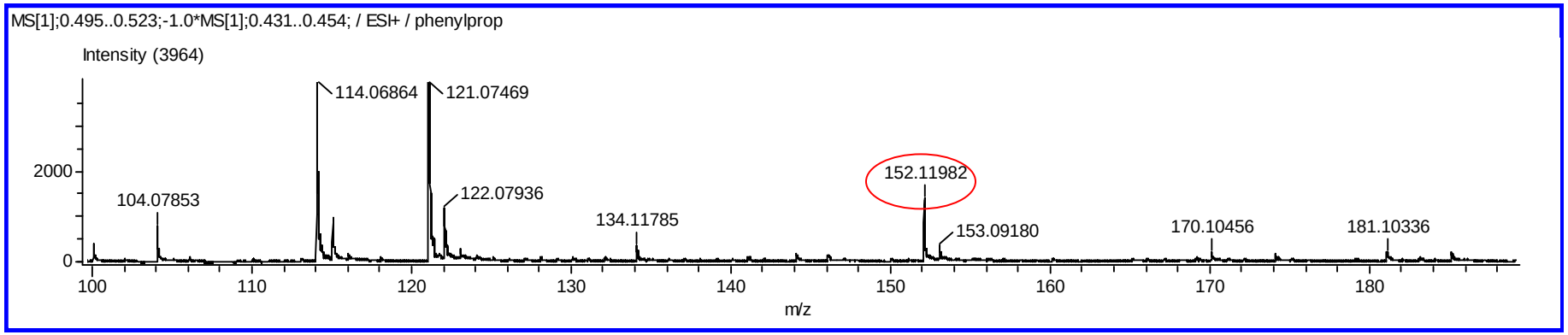


B-2. Allergy/Cold (continued)

B-2.14. Phenylpropanolamine 100 μg/mL 152.106

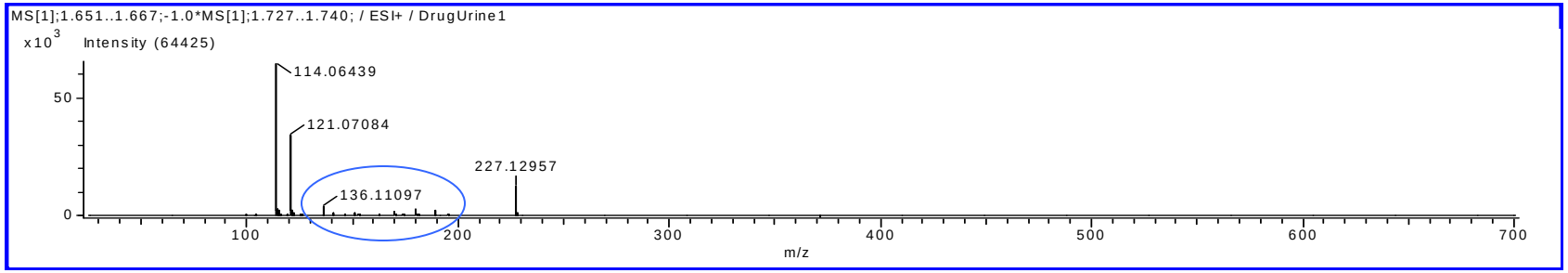


B-2.15. Phenylpropanolamine 6.25 μg/mL 152.106

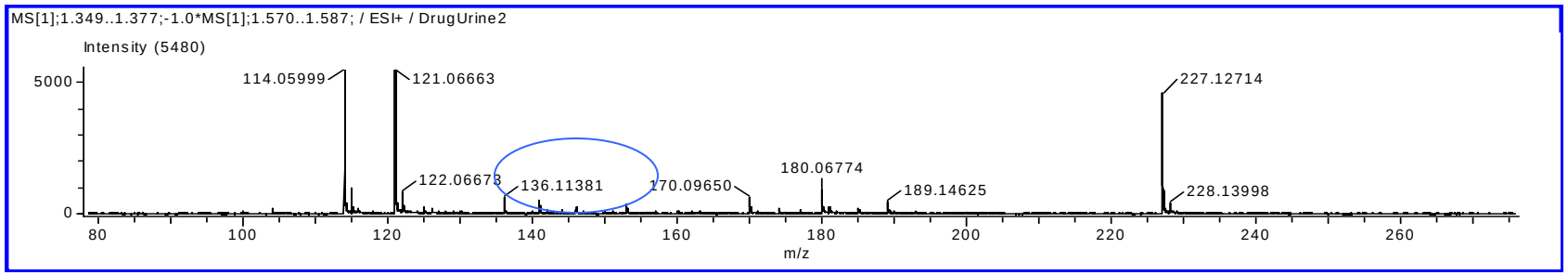


B-3. Amphetamines

B-3.1. Amphetamine 12.5 μg/mL 136.111

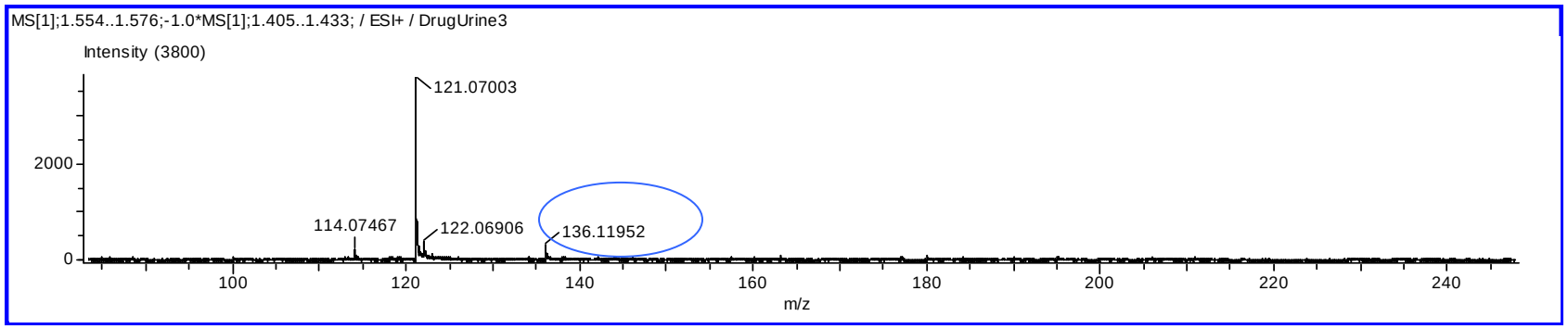


B-3.2. Amphetamine 6.25 μg/mL 136.111

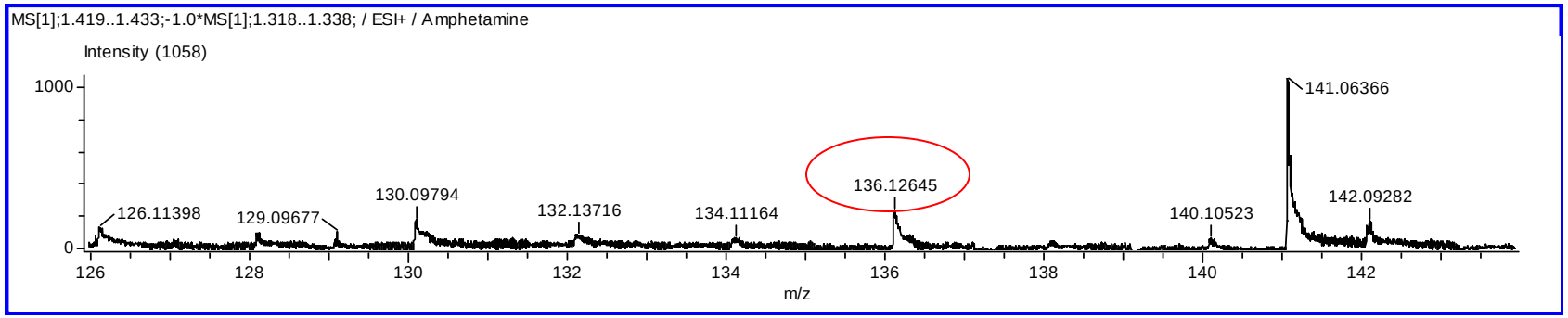


B-3. Amphetamines (continued)

B-3.3. Amphetamine 3.125 μg/mL 136.111

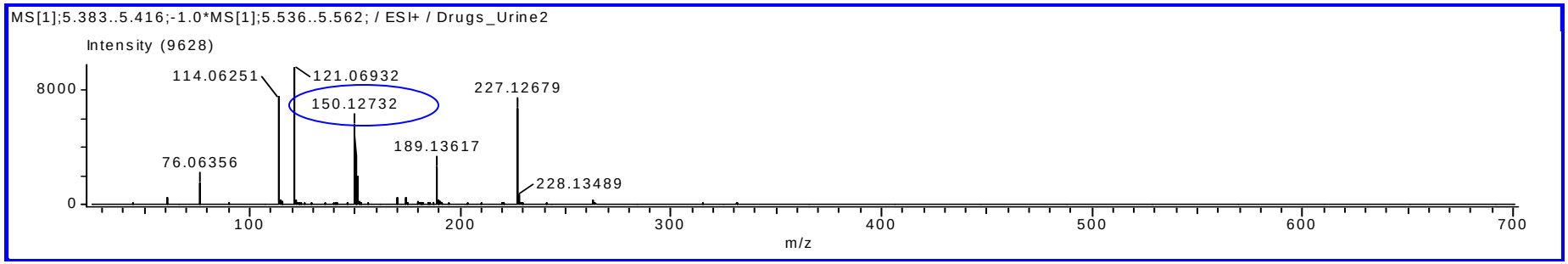


B-3.4. Amphetamine 1.56 μg/mL 136.111

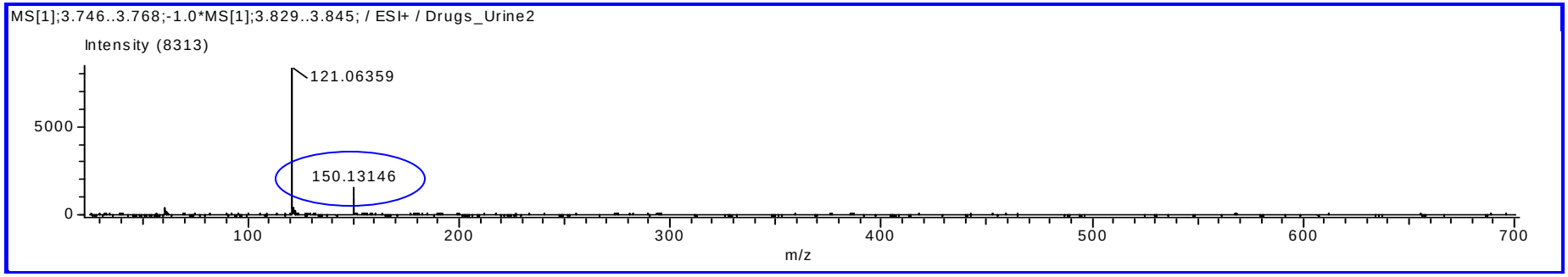


B-3. Amphetamines (continued)

B-3.5. Methamphetamine 100 μg/mL 150.127

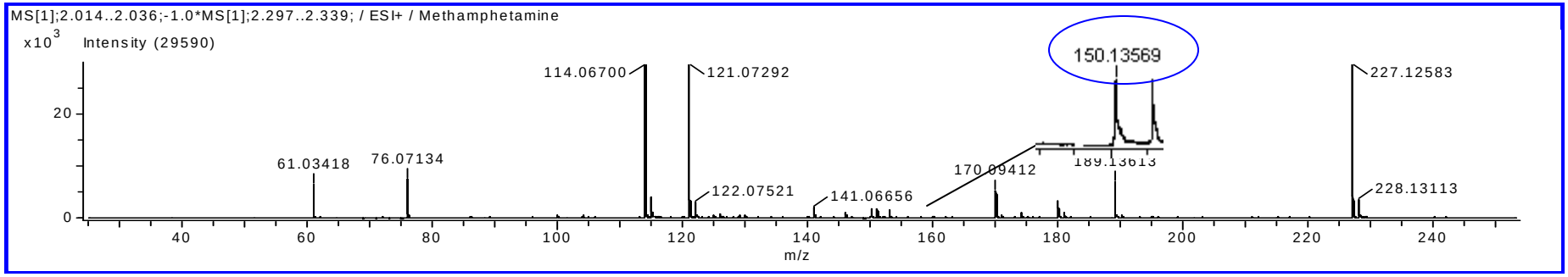


B-3.6. Methamphetamine 50 μg/mL 150.127

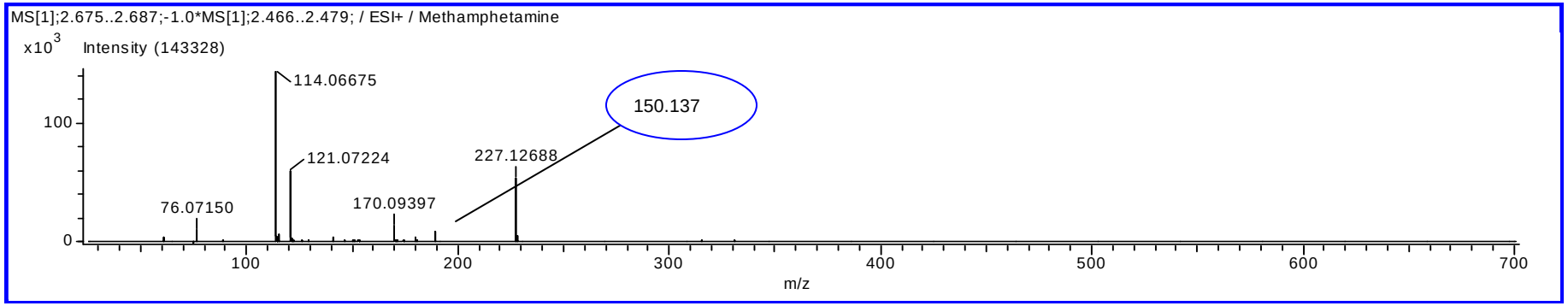


B-3. Amphetamines (continued)

B-3.7. Methamphetamine 25 μg/mL 150.127

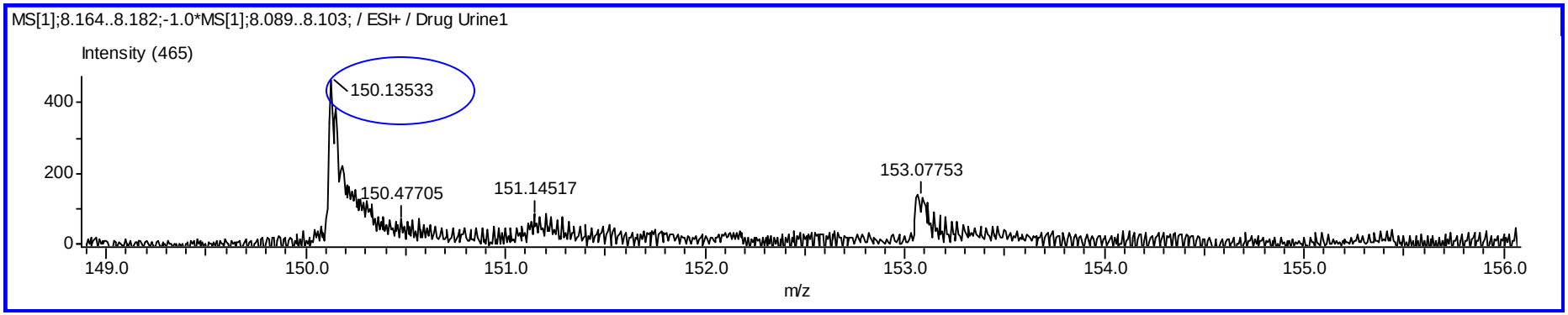


B-3.8. Methamphetamine 12.5 μg/mL 150.127

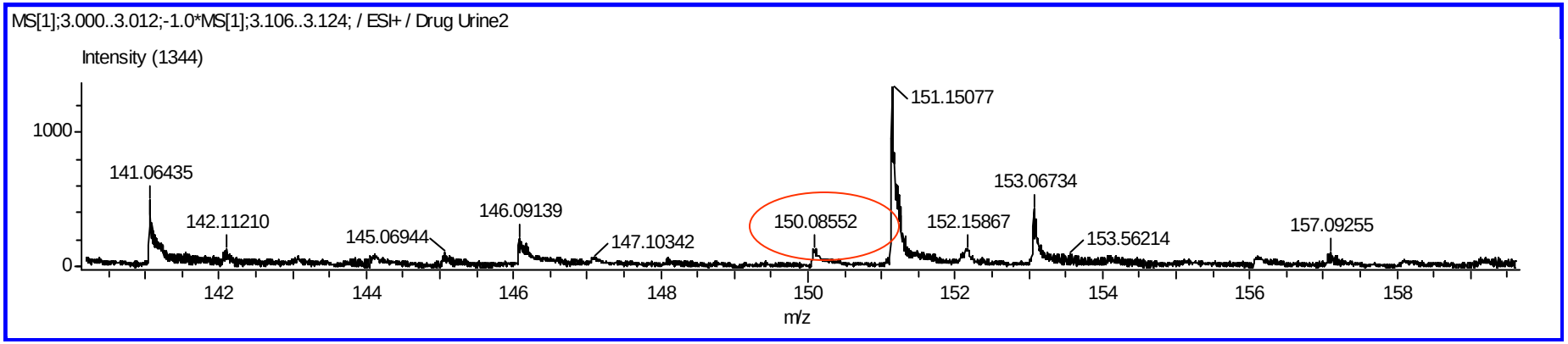


B-3. Amphetamines (continued)

B-3.9. Methamphetamine 6.25 μg/mL 150.127

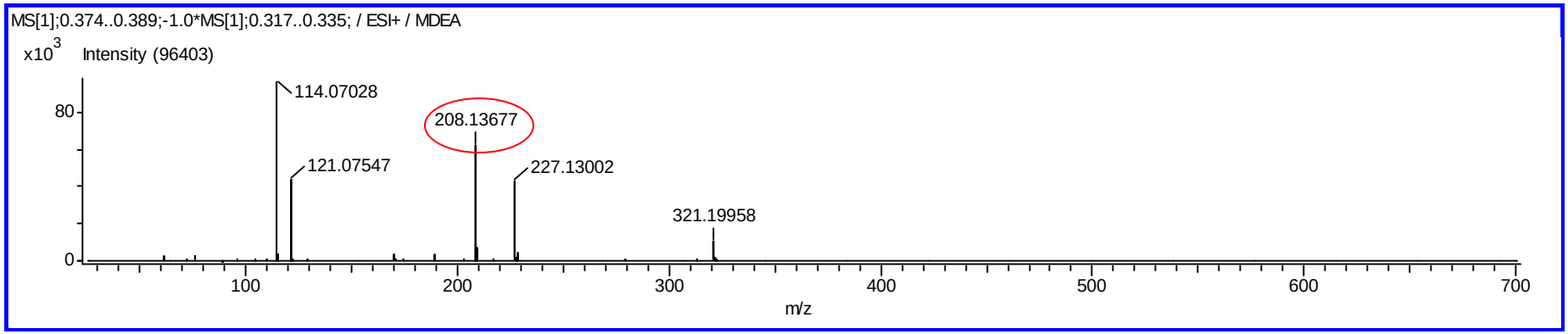


B-3.10. Methamphetamine 3.125 μg/mL 150.127

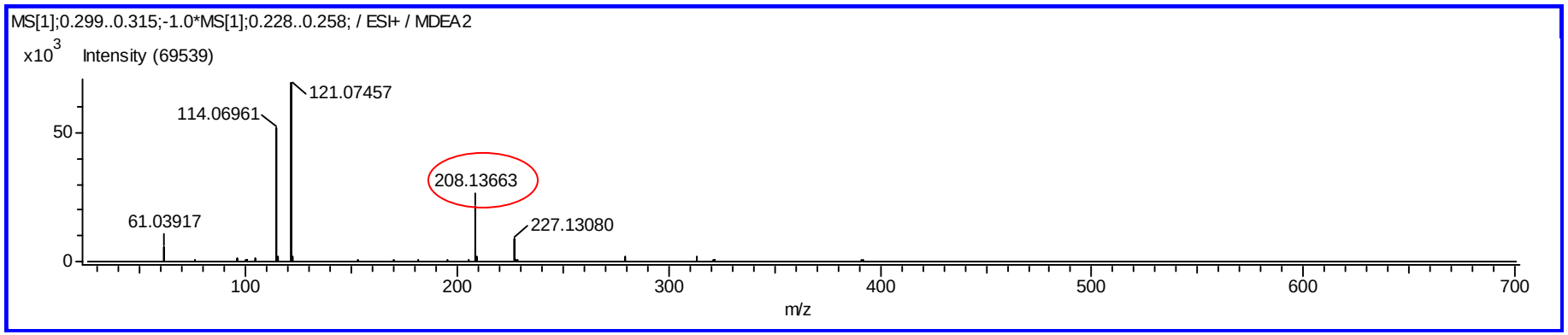


B-3. Amphetamines (continued)

B-3.11. MDEA 100 µg/mL 208.132

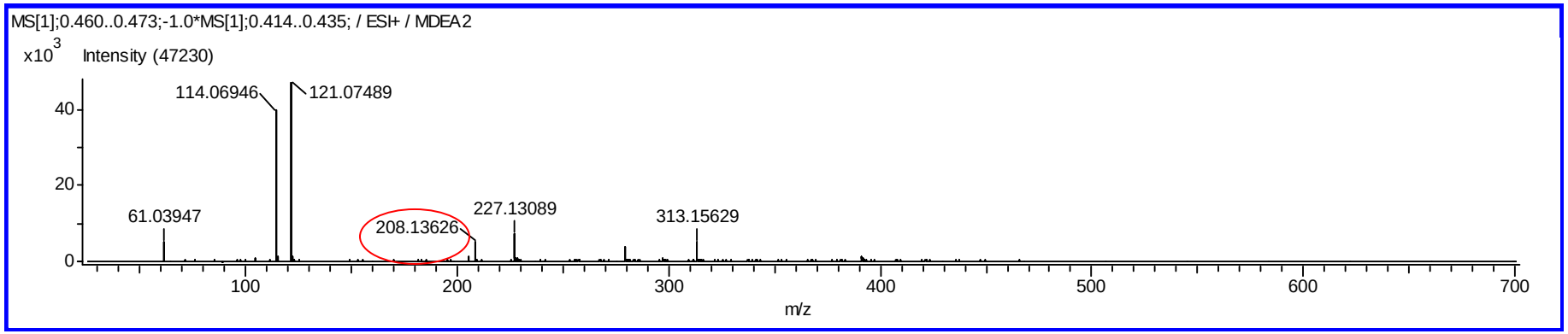


B-3.12. MDEA 25 µg/mL 208.132



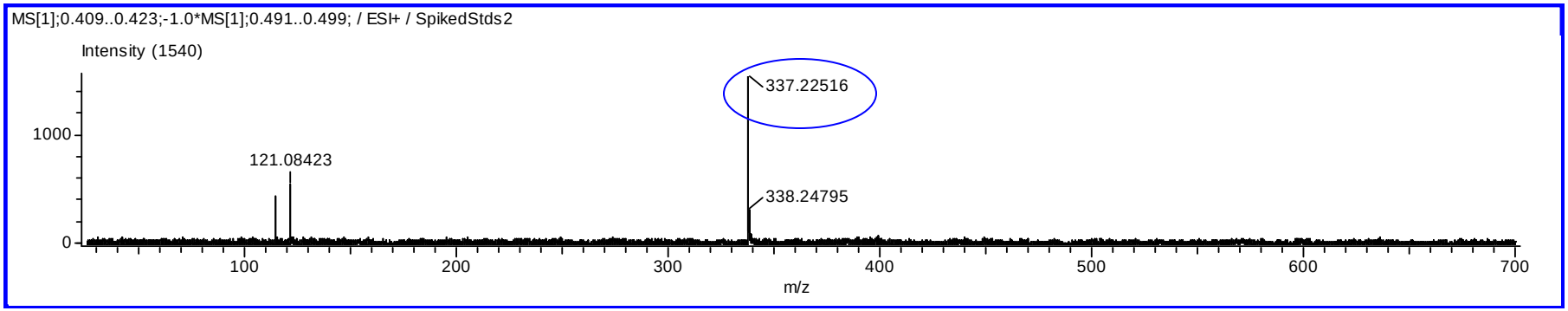
B-3. Amphetamines (continued)

B-3.13. MDEA 12.5 μg/mL 208.132

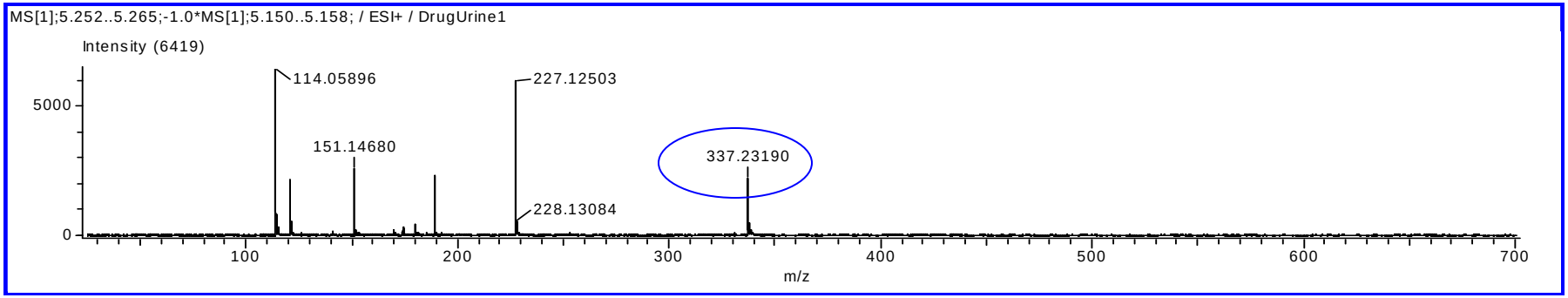


B-4. Analgesics

B-4.1. Fentanyl 45 μg/mL 337.227

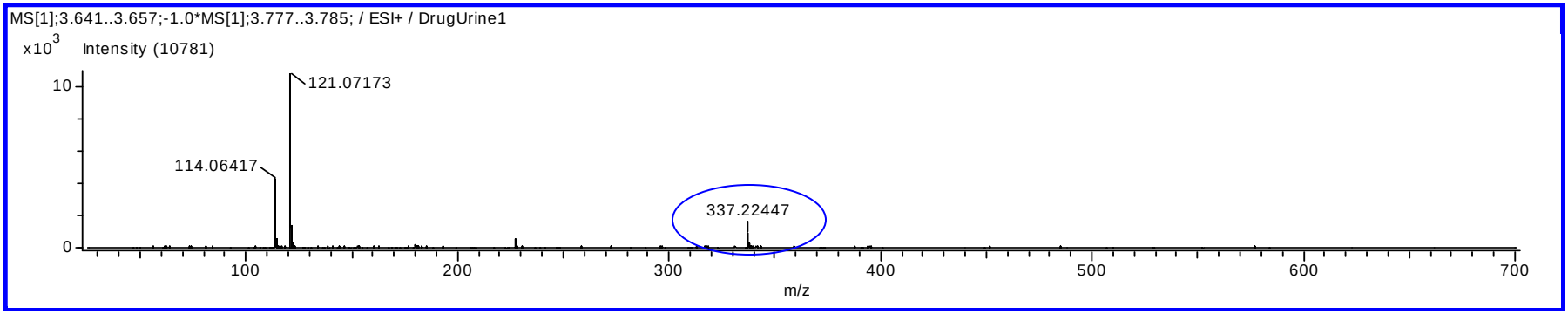


B-4.2. Fentanyl 10 μg/mL 337.227

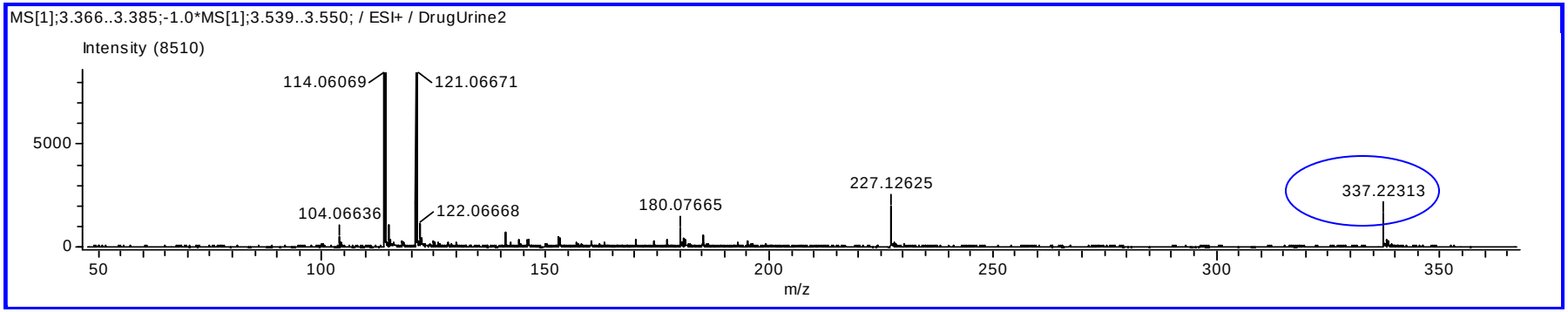


B-4. Analgesics (continued)

B-4.3. Fentanyl 5 μg/mL 337.227

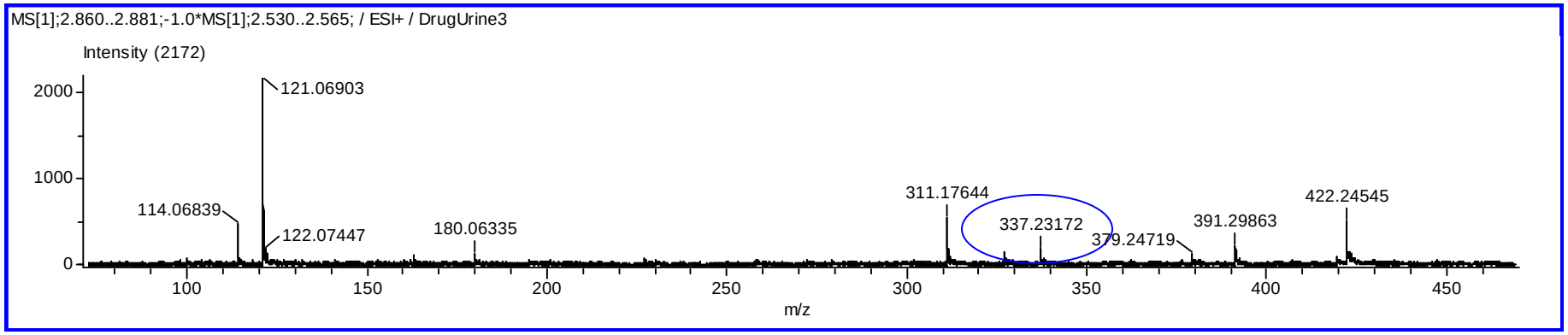


B-4.4. Fentanyl 2.50 μg/mL 337.227

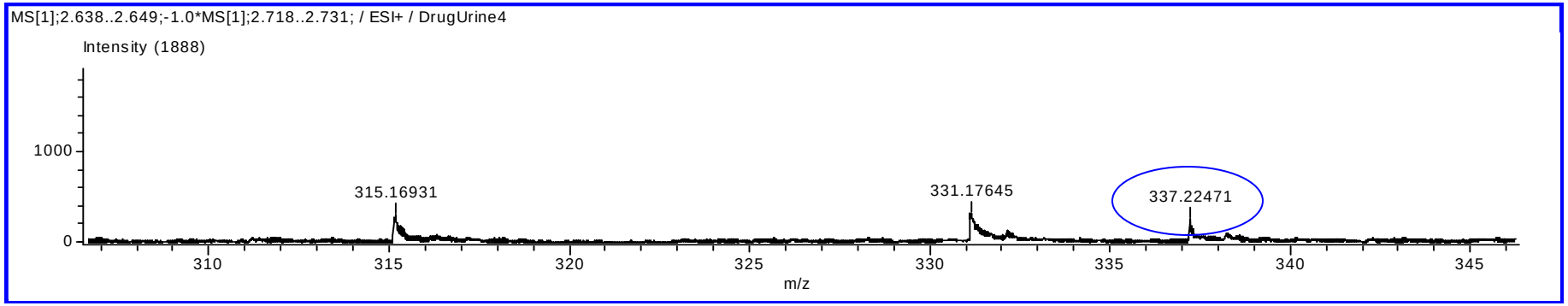


B-4. Analgesics (continued)

B-4.5. Fentanyl 1.25 μg/mL 337.227

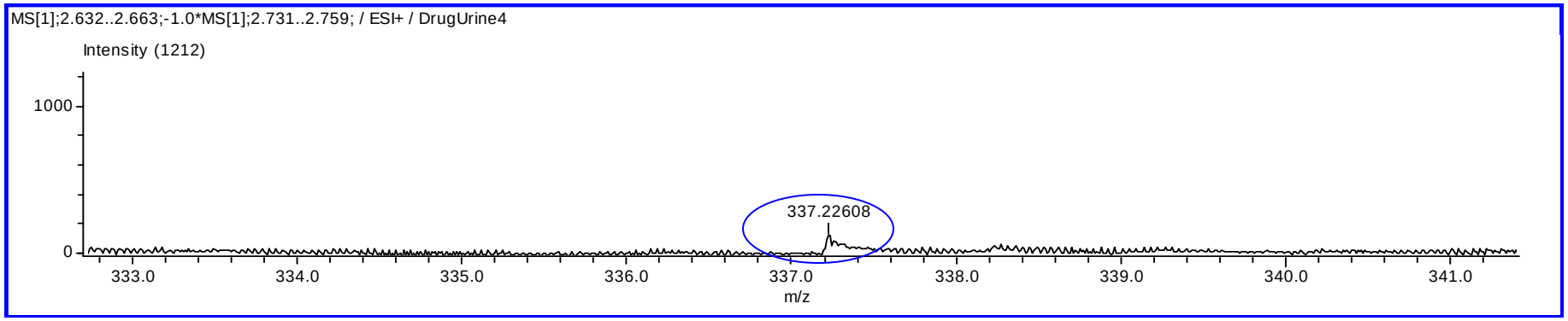


B-4.6. Fentanyl 0.62 μg/mL 337.227



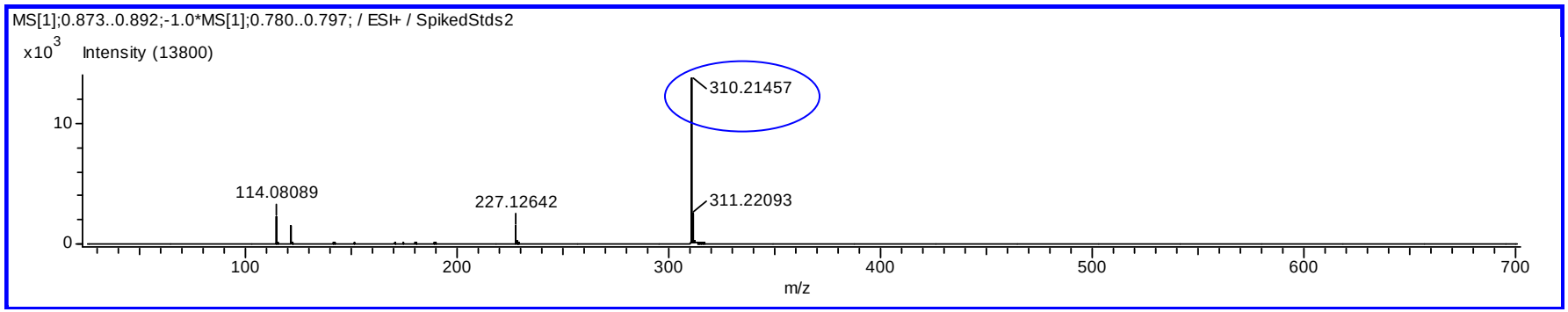
B-4. Analgesics (continued)

B-4.7. Fentanyl 0.31 μg/mL 337.227

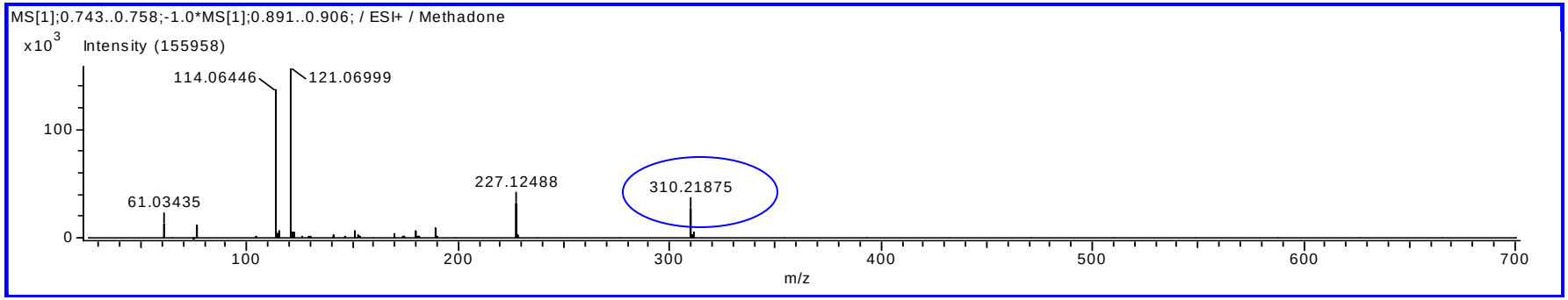


B-4. Analgesics (continued)

B-4.8. Methadone 100 μg/mL 310.216

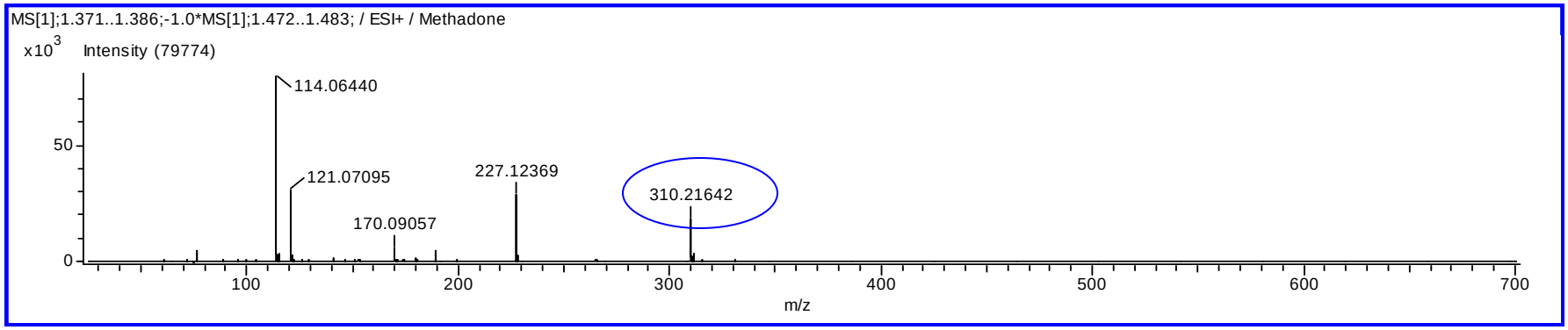


B-4.9. Methadone 25 μg/mL 310.216

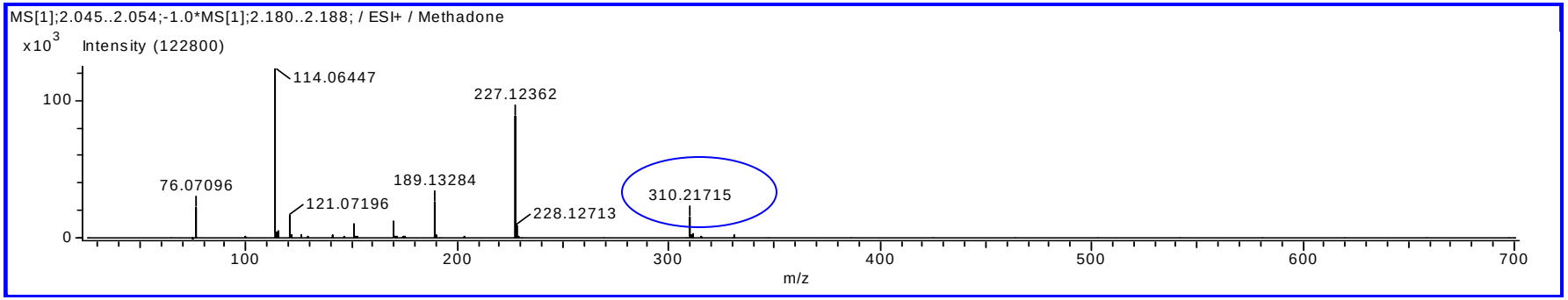


B-4. Analgesics (continued)

B-4.10. Methadone 12.5 μg/mL 310.216

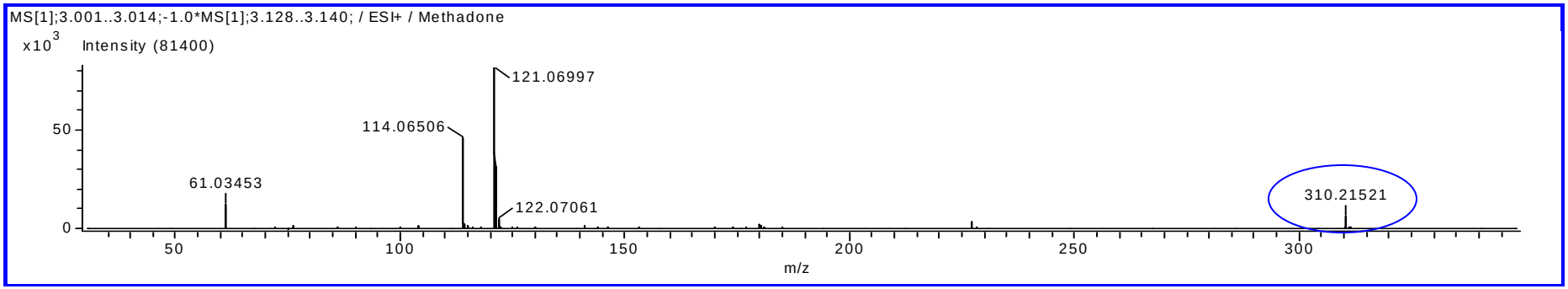


B-4.11. Methadone 6.25 μg/mL 310.216

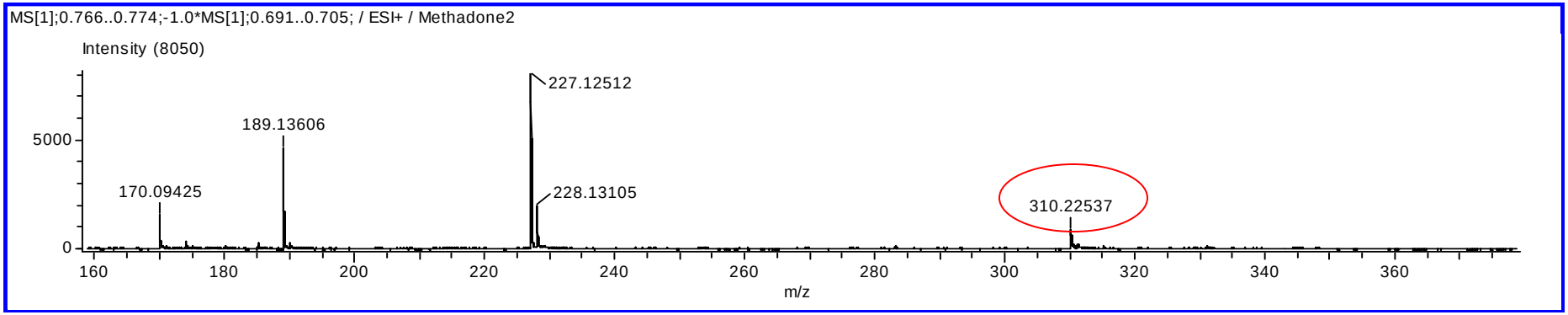


B-4. Analgesics (continued)

B-4.12. Methadone 3.125 μg/mL 310.216

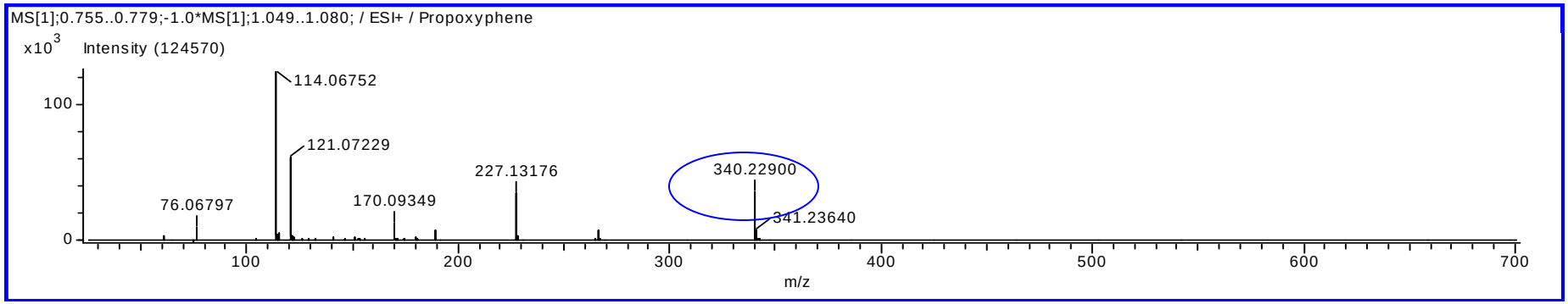


B-4.13. Methadone 1.56 μg/mL 310.216

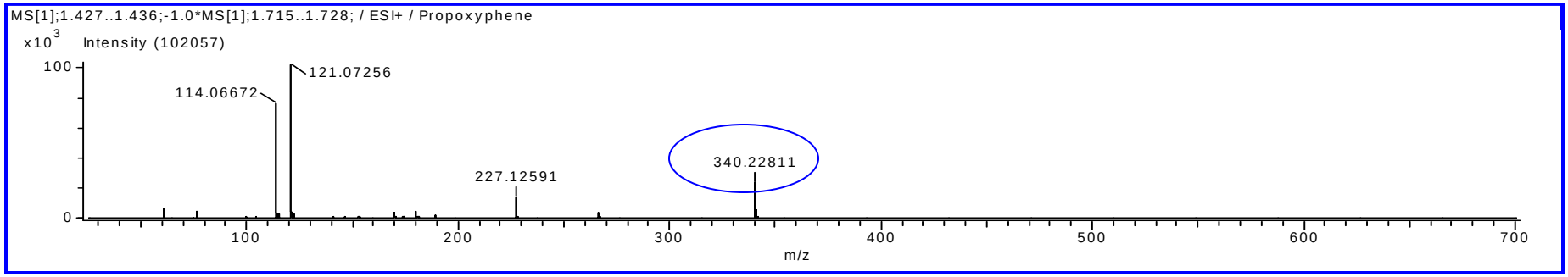


B-4. Analgesics (continued)

B-4.14. Propoxyphene 50 µg/mL 340.226

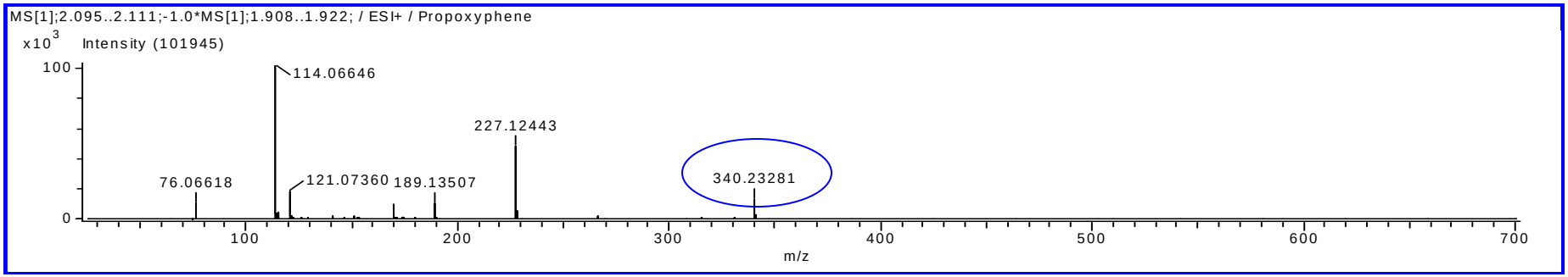


B-4.15. Propoxyphene 25 µg/mL 340.226

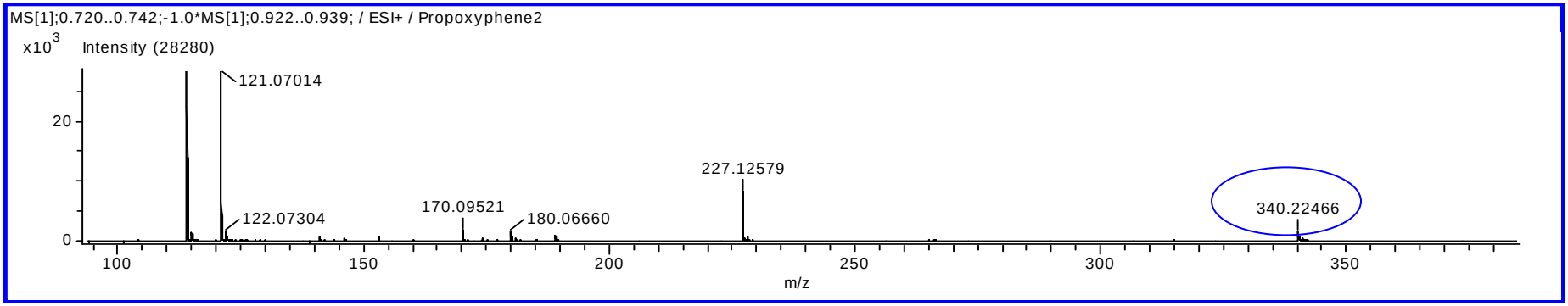


B-4. Analgesics (continued)

B-4.16. Propoxyphene 12.5 μg/mL 340.226

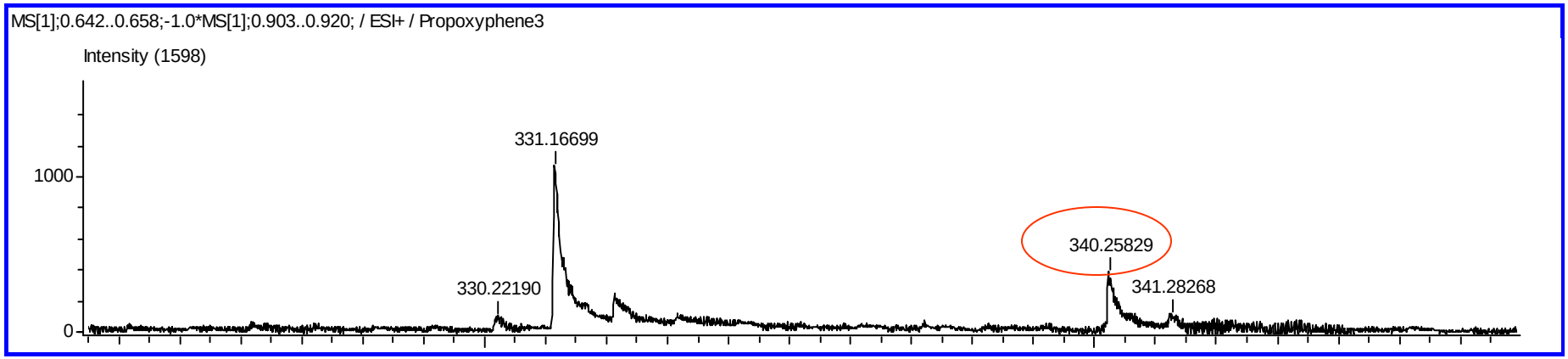


B-4.17. Propoxyphene 1.56 μg/mL 340.226



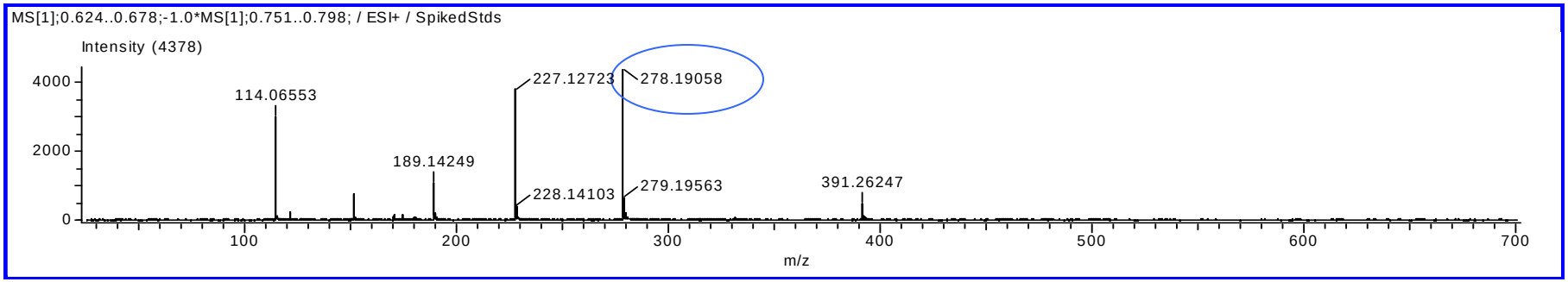
B-4. Analgesics (continued)

B-4.18. Propoxyphene 0.78 μg/mL 340.226

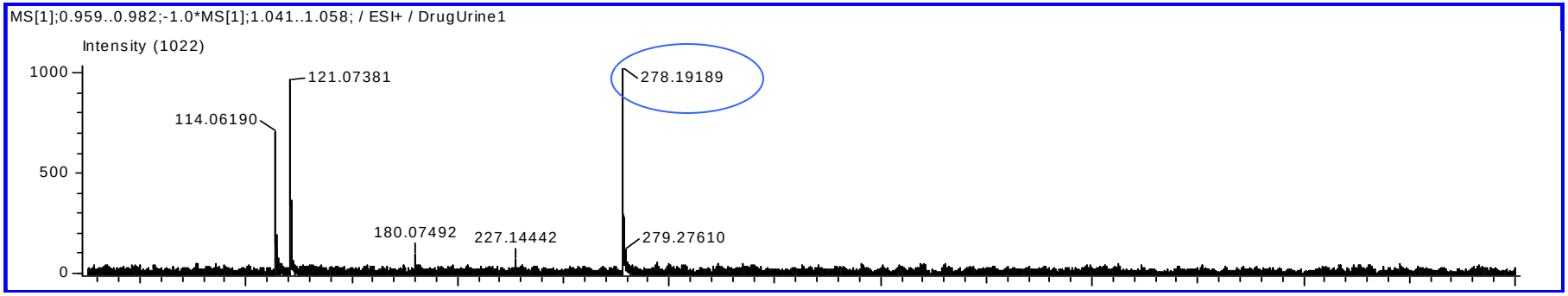


B-5. Antidepressants

B-5.1. Amitriptyline 100 μg/mL 278.190

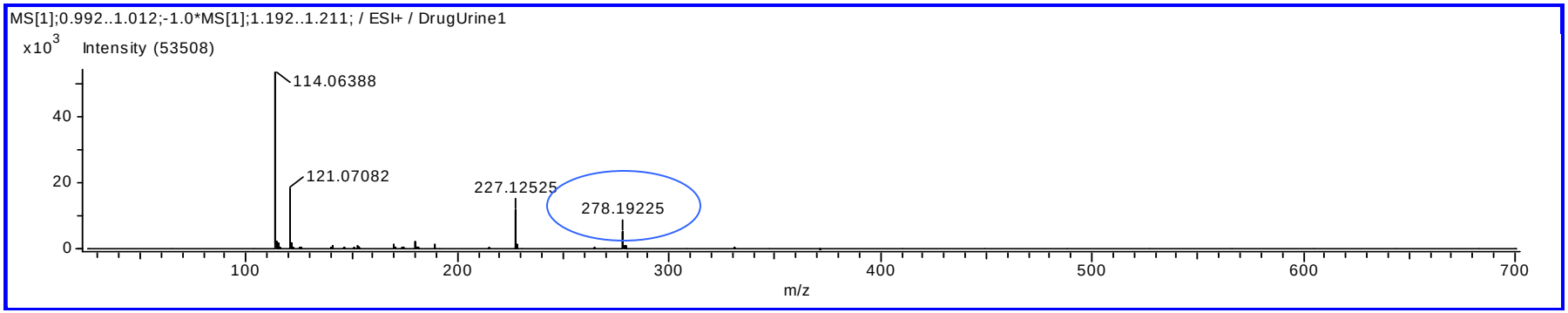


B-5.2. Amitriptyline 25 μg/mL 278.190

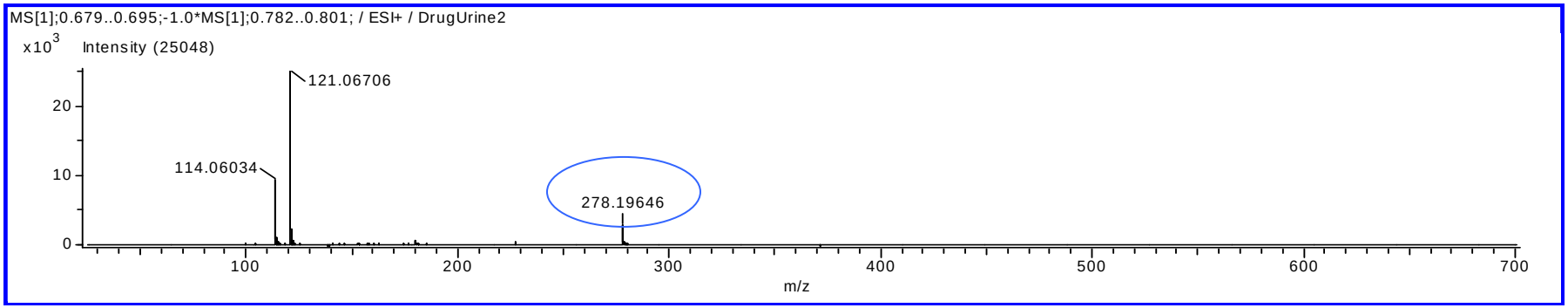


B-5. Antidepressants (continued)

B-5.3. Amitriptyline 12.5 μg/mL 278.190

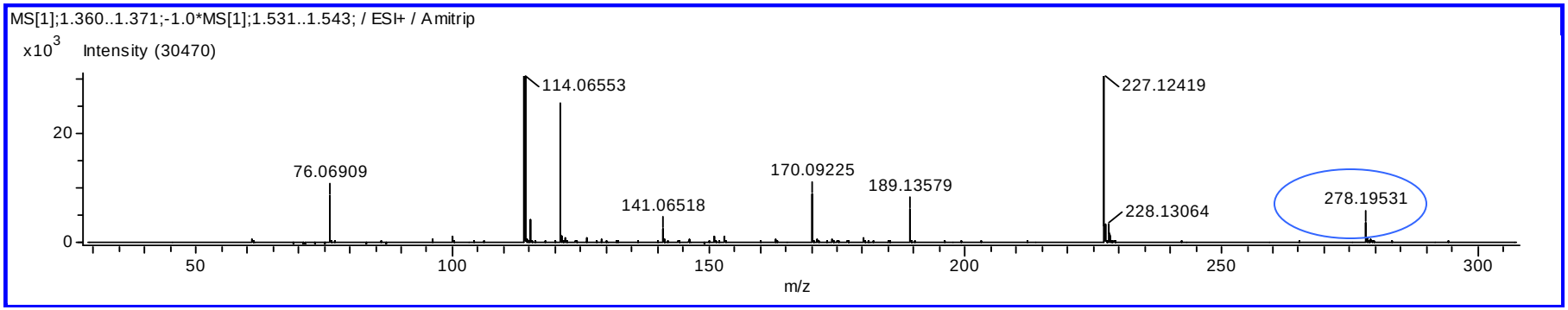


B-5.4. Amitriptyline 6.25 μg/mL 278.190

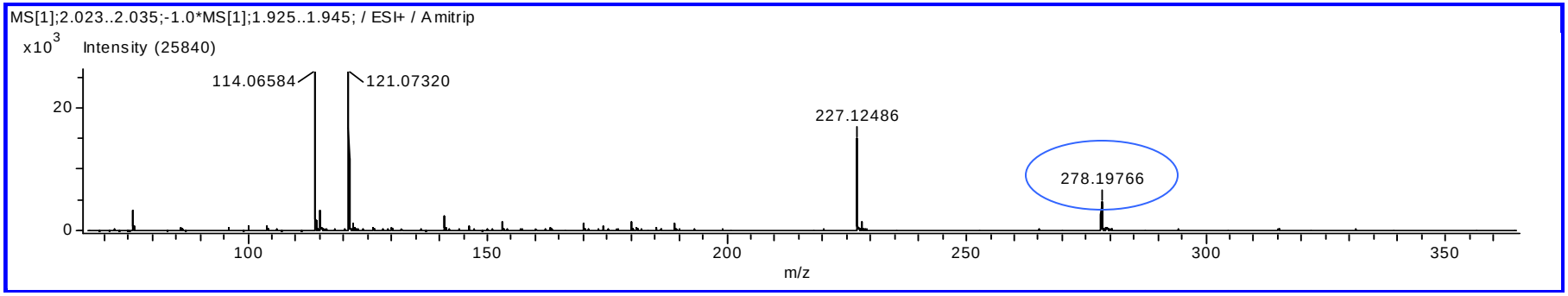


B-5. Antidepressants (continued)

B-5.5. Amitriptyline 3.125 μg/mL 278.190

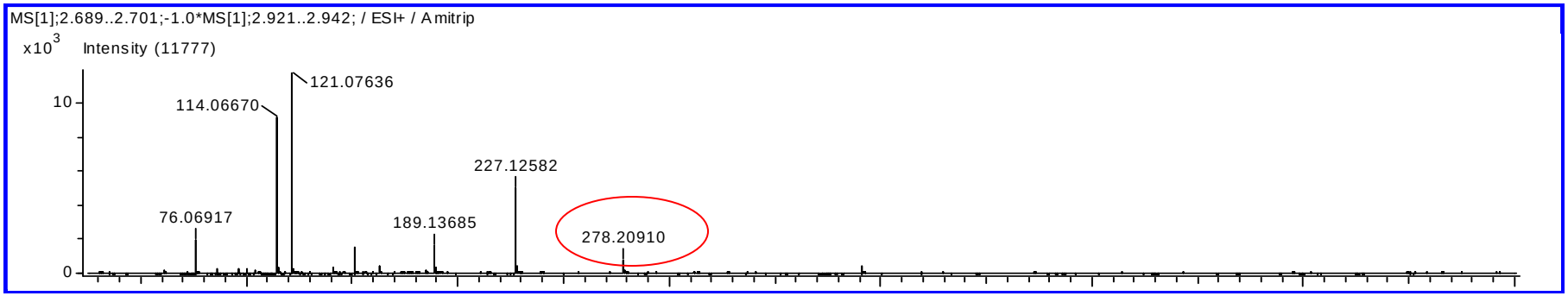


B-5.6. Amitriptyline 1.56 μg/mL 278.190



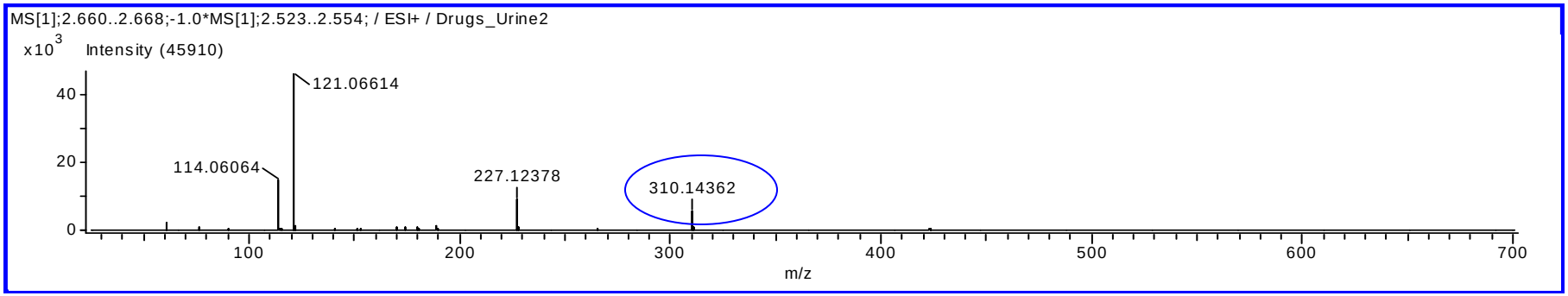
B-5. Antidepressants (continued)

B-5.7. Amitriptyline 0.78 μg/mL 278.190

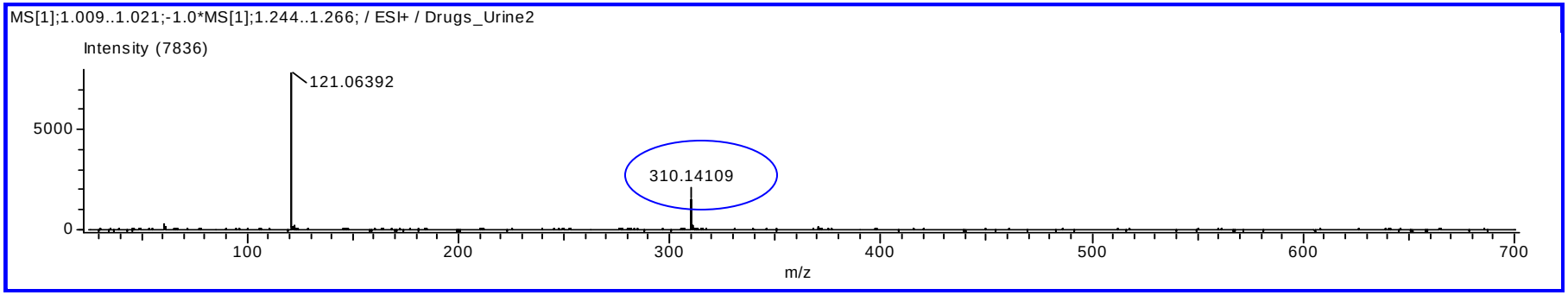


B-5. Antidepressants (continued)

B-5.8. Fluoxetine 100 μg/mL 310.141

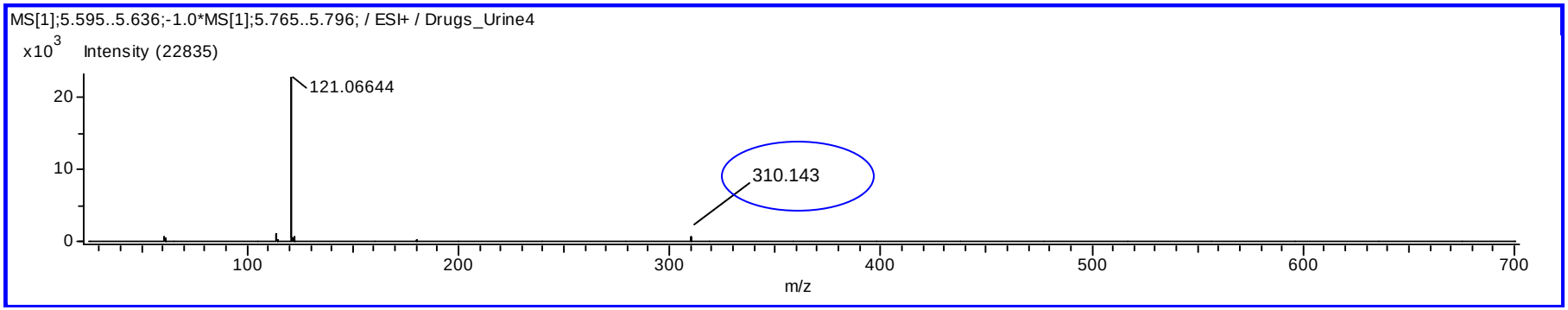


B-5.9. Fluoxetine 50 μg/mL 310.141

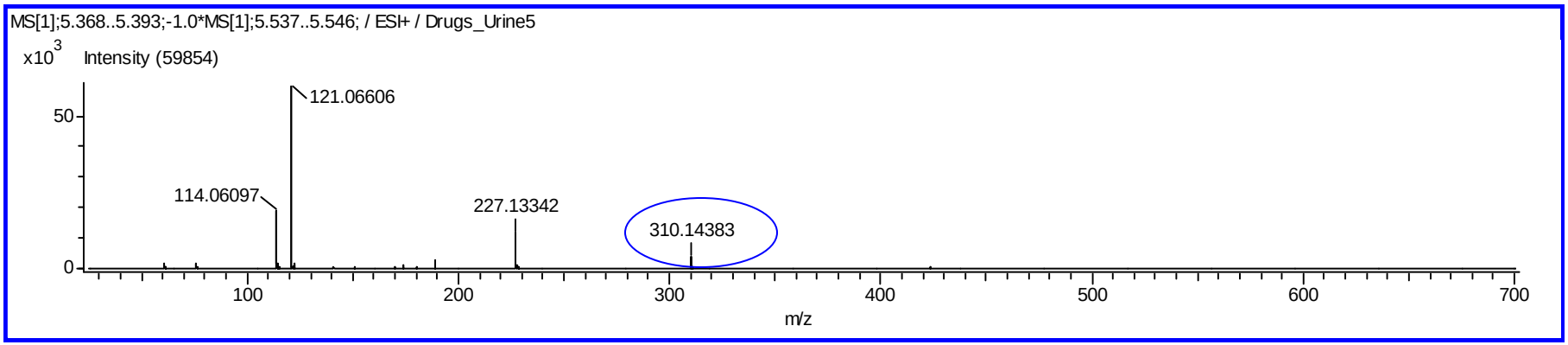


B-5. Antidepressants (continued)

B-5.10. Fluoxetine 25 μg/mL 310.141

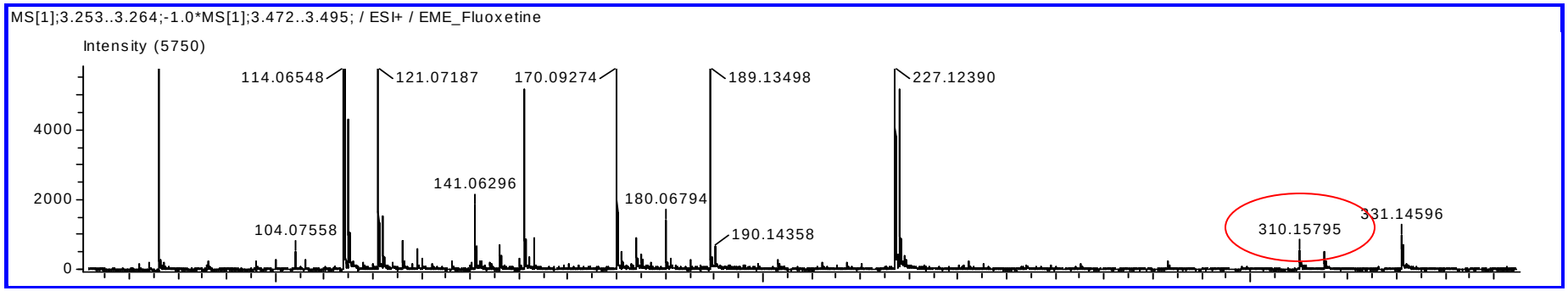


B-5.11. Fluoxetine 12.5 μg/mL 310.141



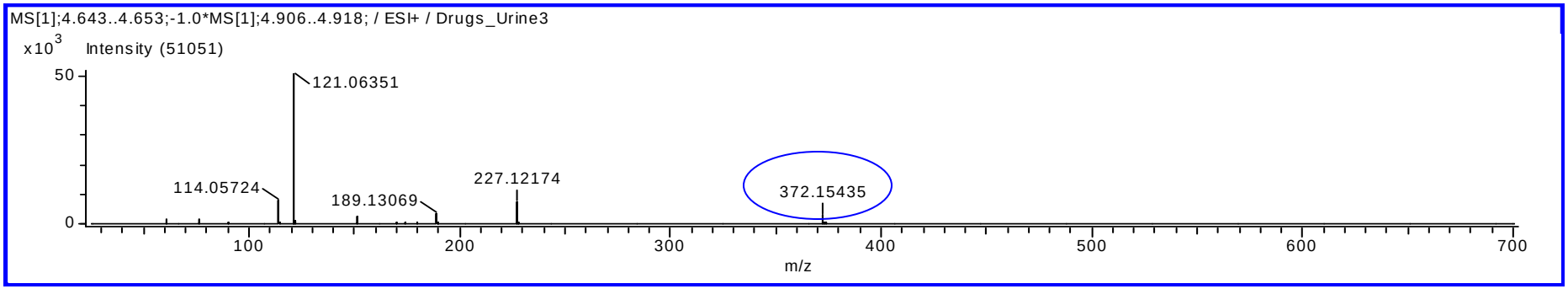
B-5. Antidepressants (continued)

B-5.12. Fluoxetine 6.25 μg/mL 310.141

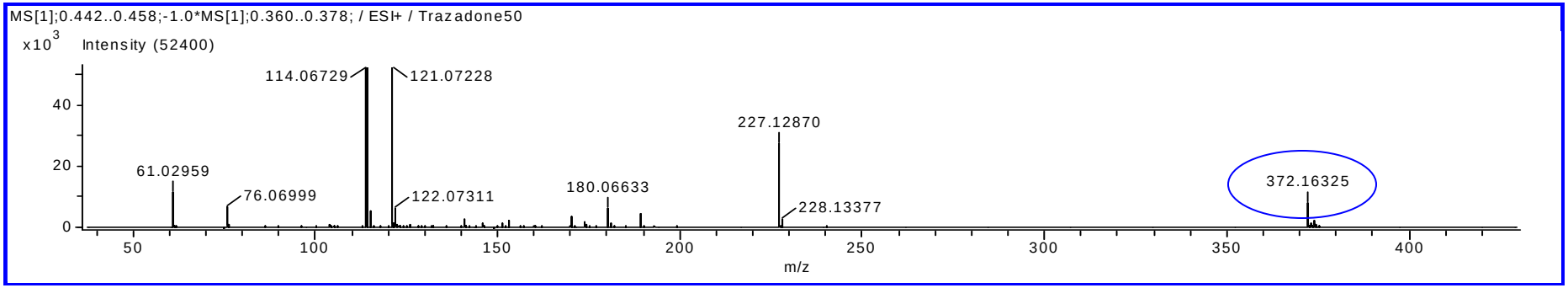


B-5. Antidepressants (continued)

B-5.13. Trazodone 100 μg/mL 372.158

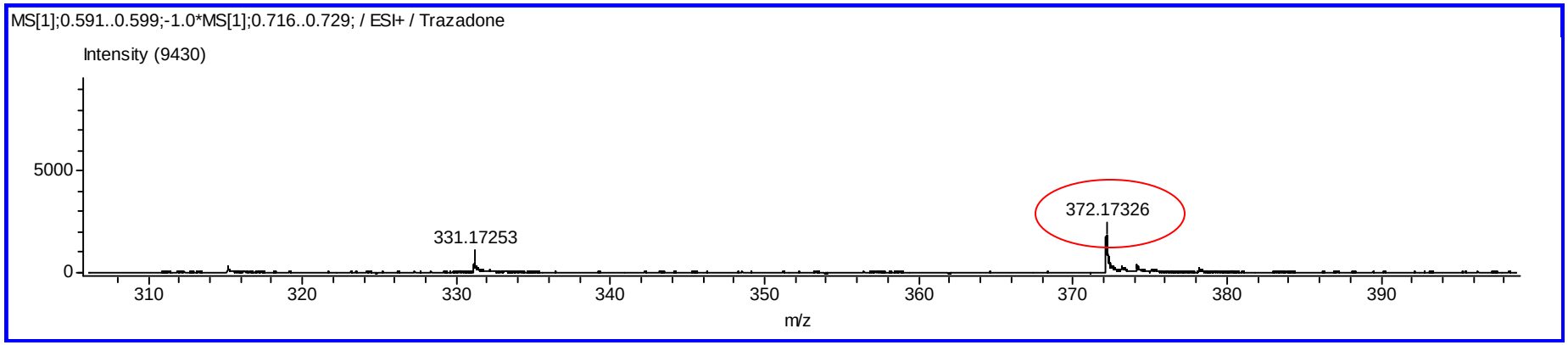


B-5.14. Trazodone 50 μg/mL 372.158



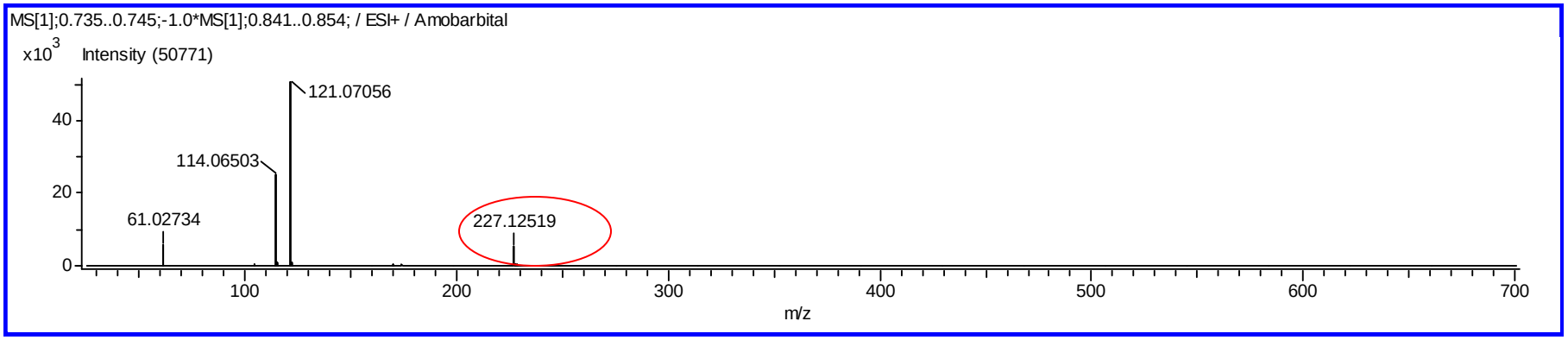
B-5. Antidepressants (continued)

B-5.15. Trazodone 25 μg/mL 372.158

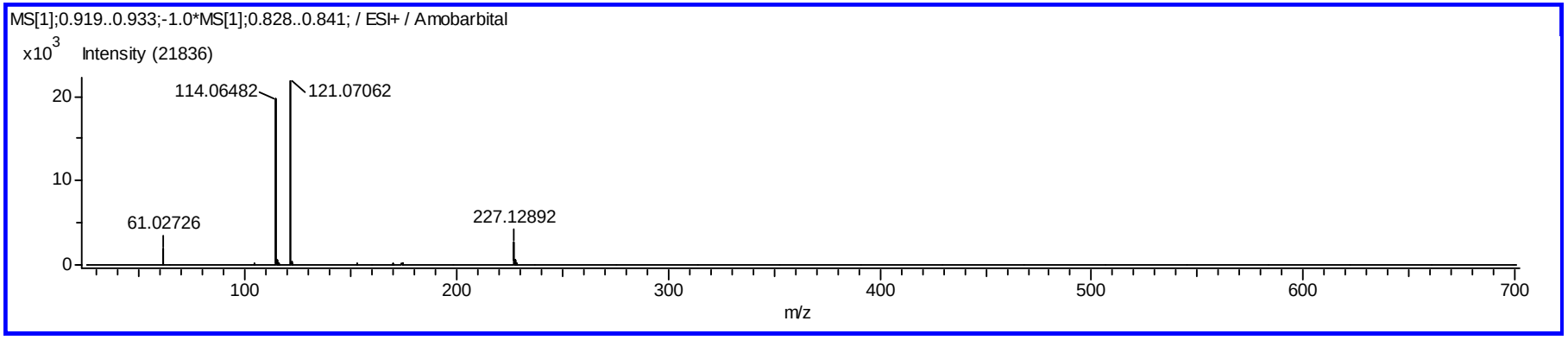


B-6. Barbiturates

B-6.1. Amobarbital 50 μg/mL 227.138*



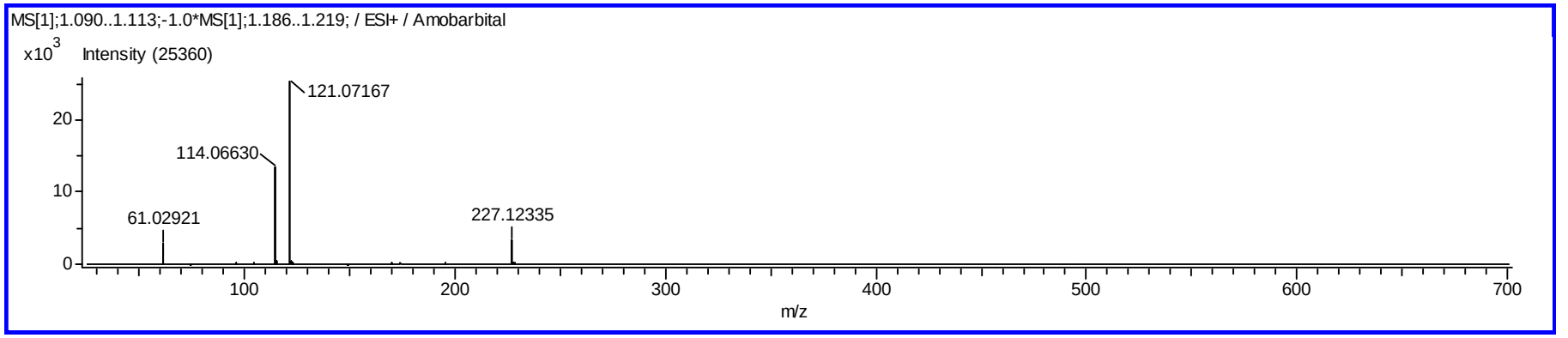
B-6.2. Amobarbital 25 μg/mL 227.138



***Creatinine dimer M+H is close to that of pentobarbital and amobarbital, making them indistinguishable.**

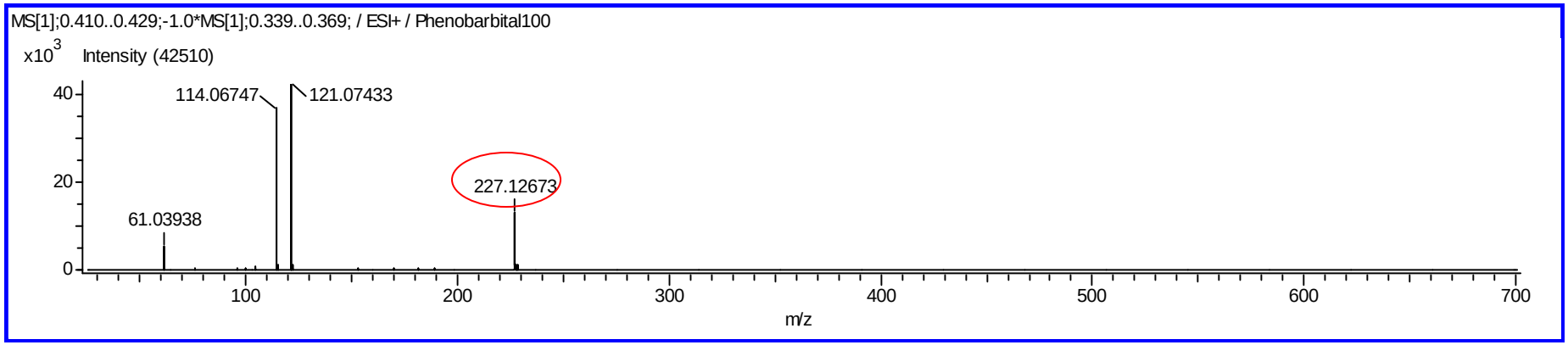
B-6. Barbiturates (continued)

B-6.3. Amobarbital 12.5 μg/mL 227.138

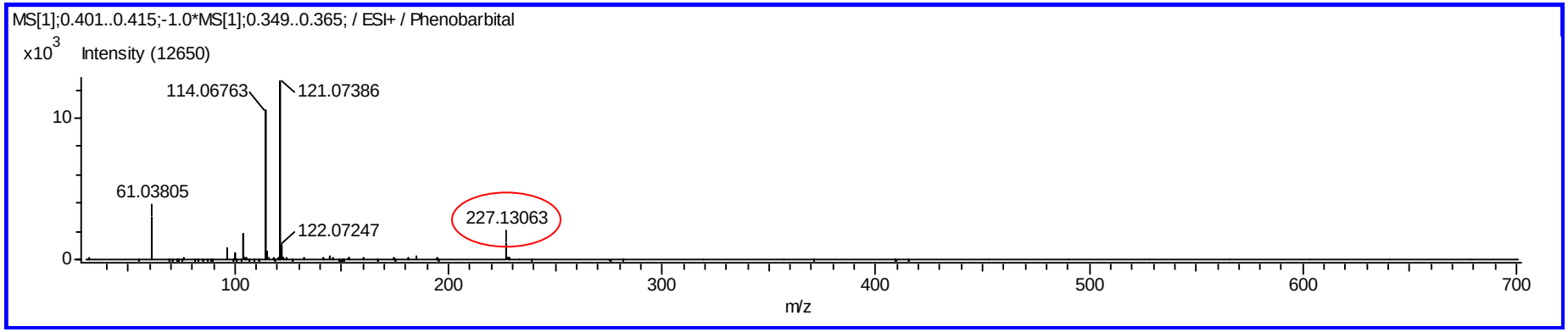


B-6. Barbiturates (continued)

B-6.4. Pentobarbital 100 μg/mL 227.138*



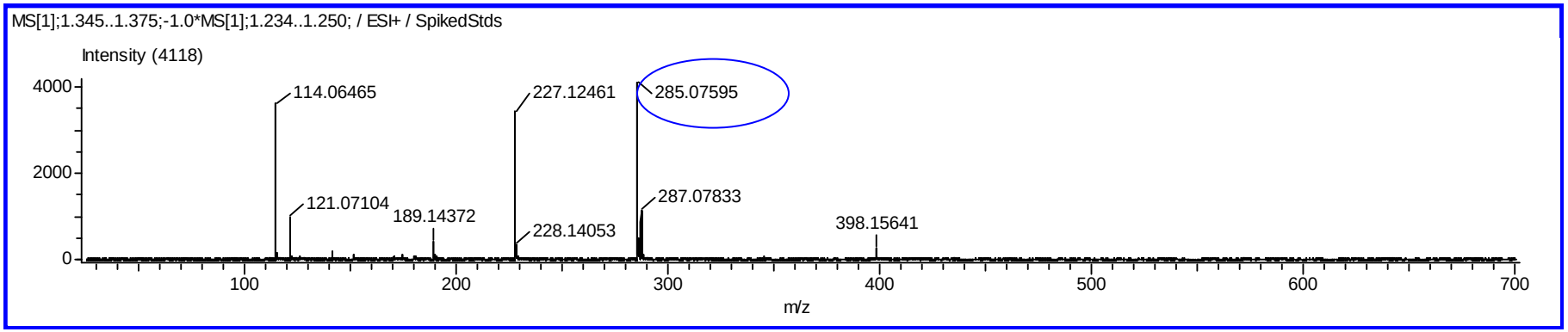
B-6.5. Pentobarbital 6.25 μg/mL 227.138



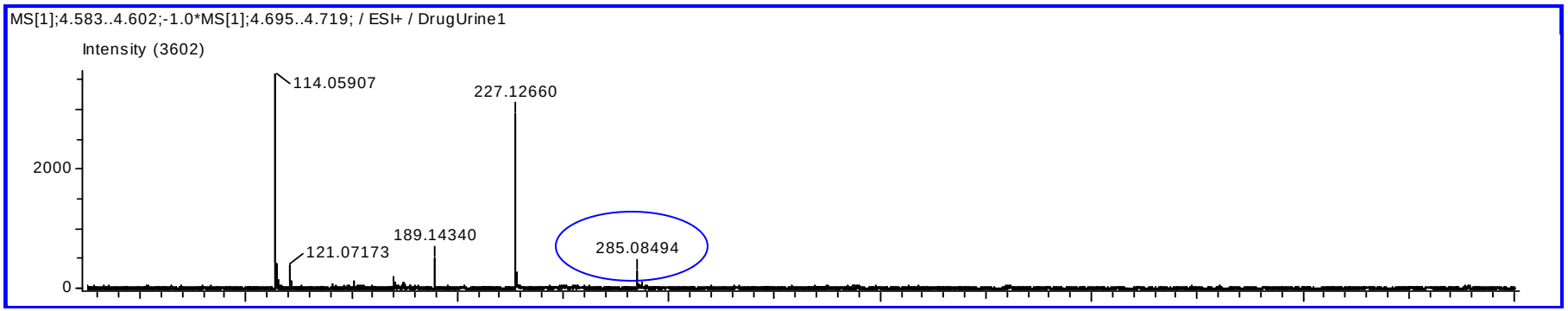
***Creatinine dimer M+H is close to that of pentobarbital and amobarbital, making them indistinguishable.**

B-7. Benzodiazepines

B-7.1. Diazepam 100 μg/mL 285.078

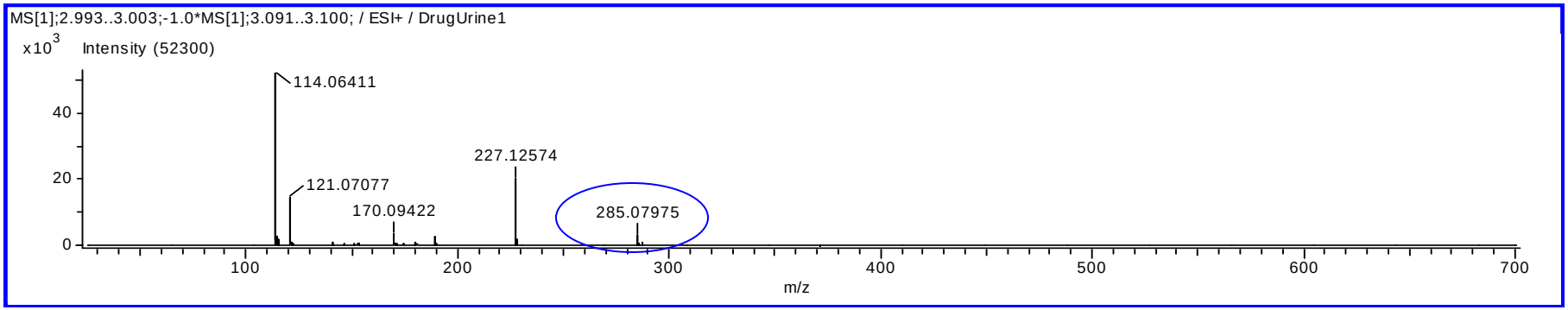


B-7.2. Diazepam 25 μg/mL 285.078

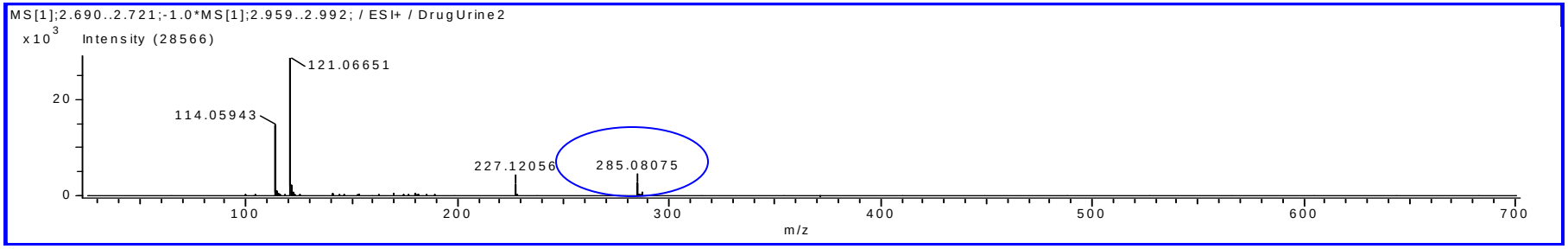


B-7. Benzodiazepines (continued)

B-7.3. Diazepam 12.5 μg/mL 285.078

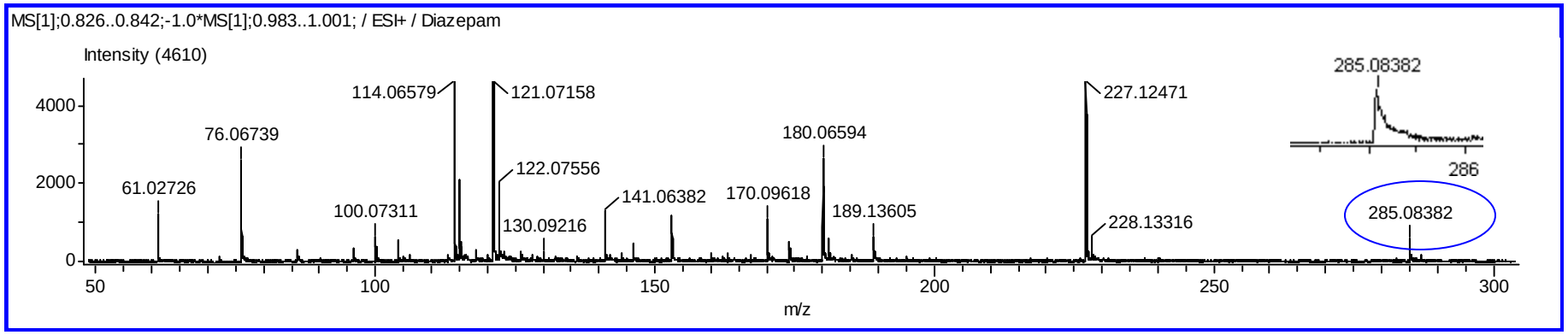


B-7.4. Diazepam 6.25 μg/mL 285.078

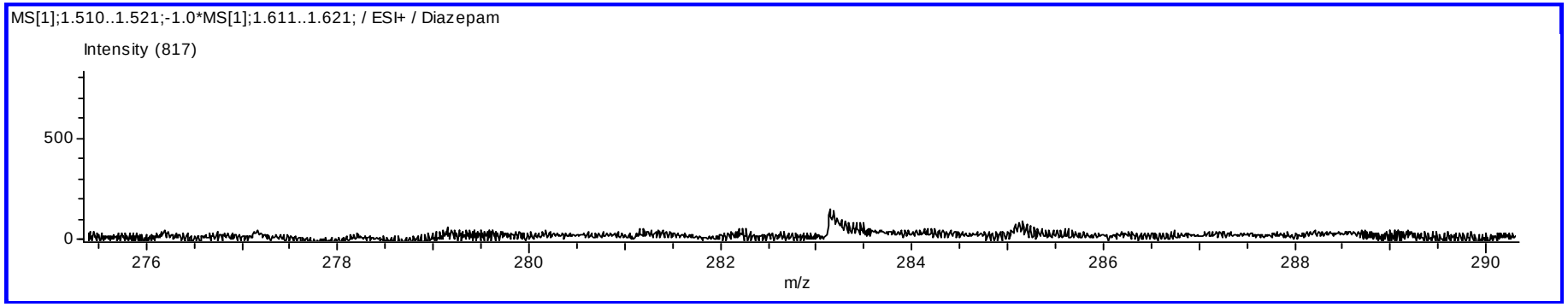


B-7. Benzodiazepines (continued)

B-7.5. Diazepam 3.125 μg/mL 285.078

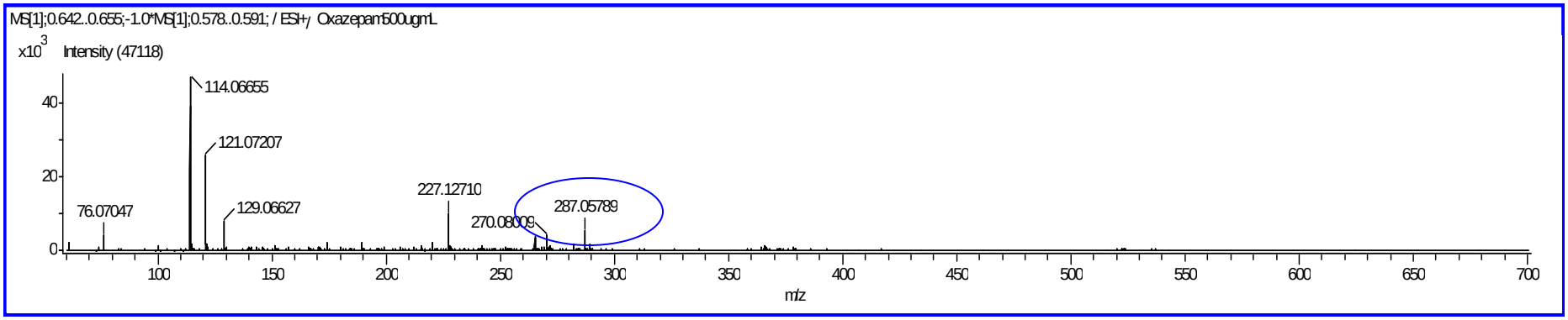


B-7.6. Diazepam 1.56 μg/mL 285.078

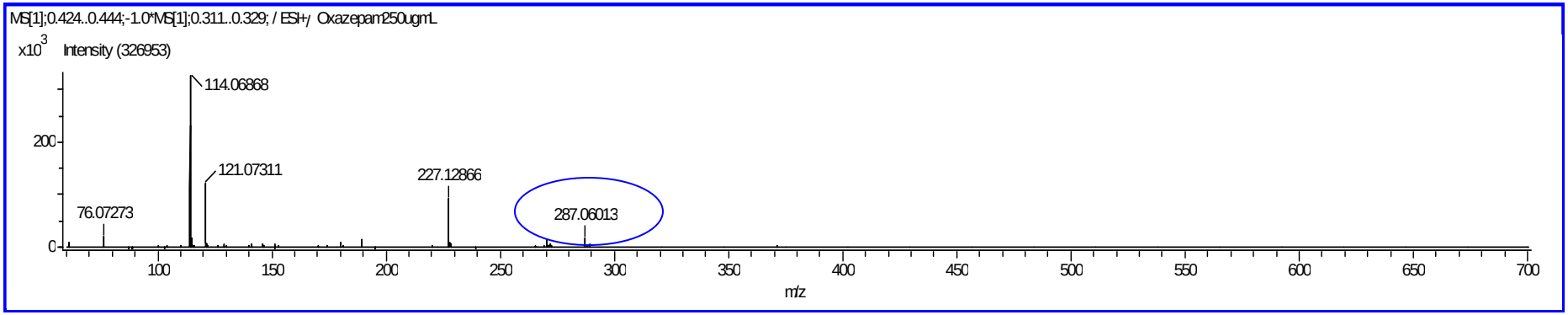


B-7. Benzodiazepines (continued)

B-7.7. Oxazepam 500 µg/mL 287.057

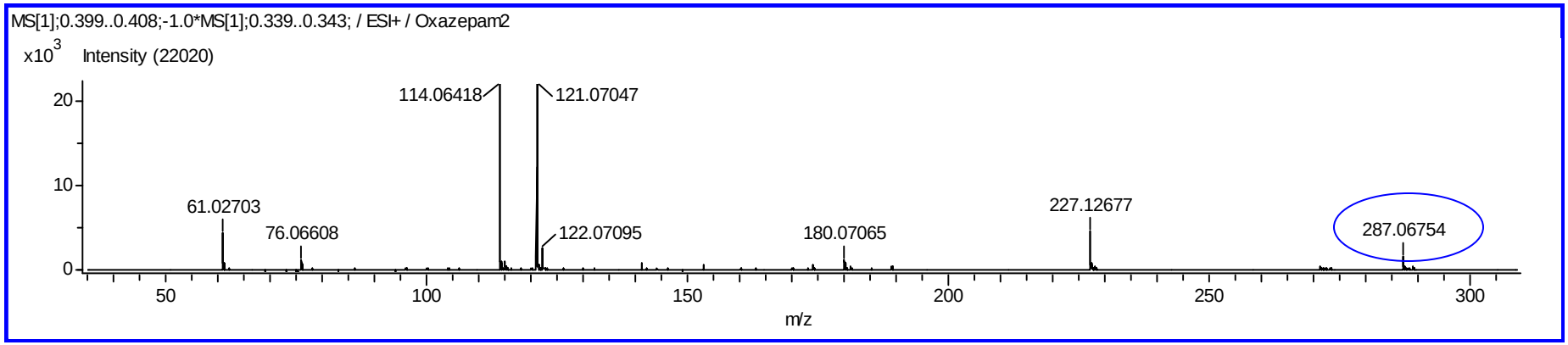


B-7.8. Oxazepam 250 µg/mL 287.057

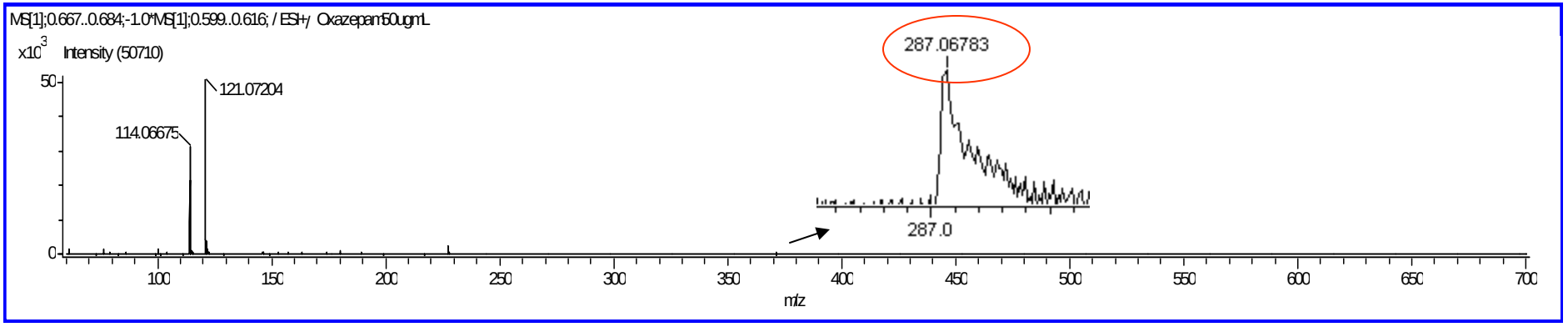


B-7. Benzodiazepines (continued)

B-7.9. Oxazepam 100 μg/mL 287.057

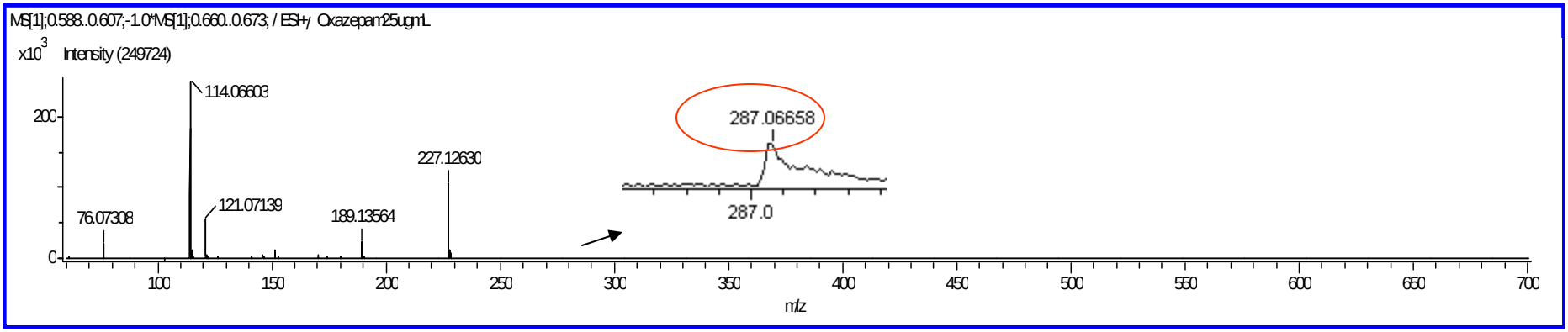


B-7.10. Oxazepam 50 μg/mL 287.057



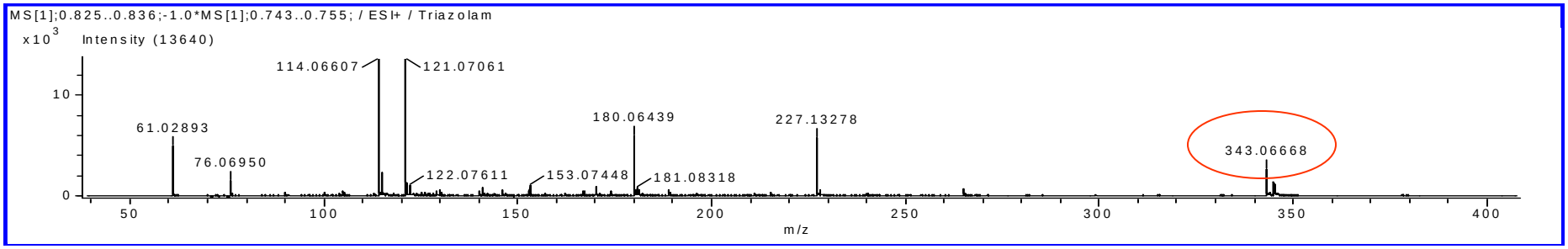
B-7. Benzodiazepines (continued)

B-7.11. Oxazepam 25 μg/mL 287.057

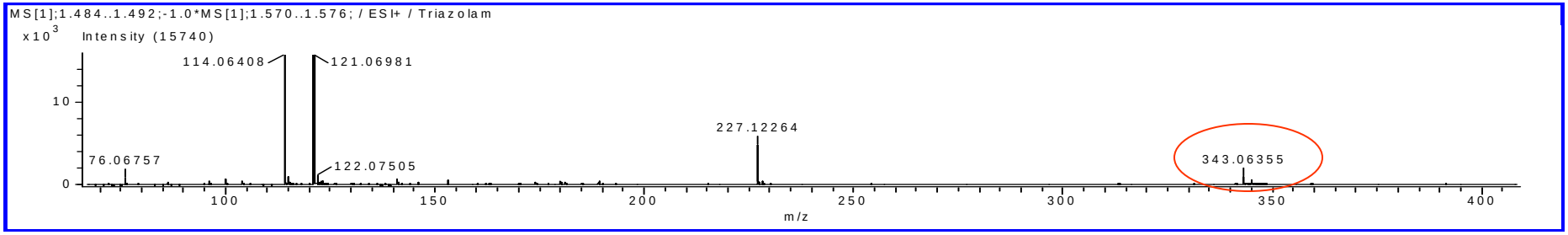


B-7. Benzodiazepines (continued)

B-7.12. Triazolam 25 μg/mL 343.050

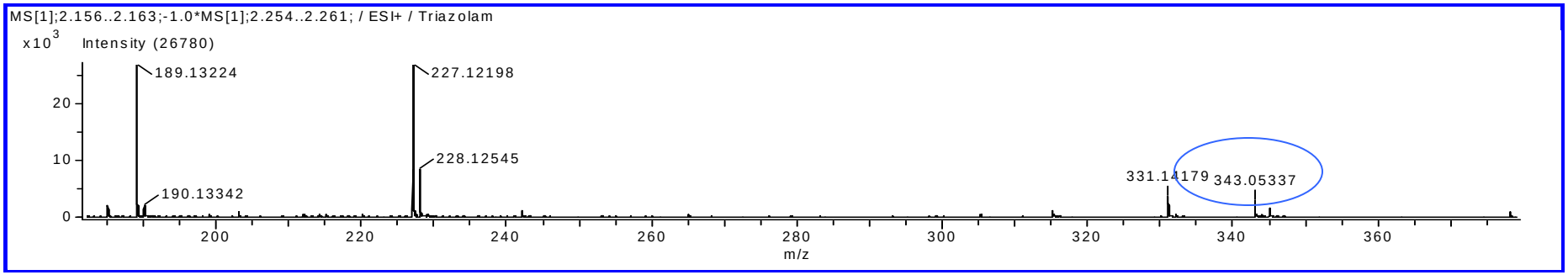


B-7.13. Triazolam 12.5 μg/mL 343.050

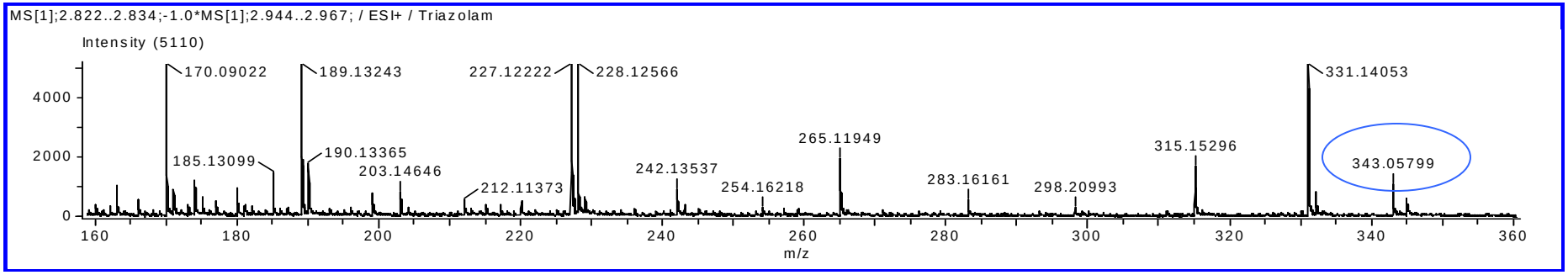


B-7. Benzodiazepines (continued)

B-7.14. Triazolam 6.25 μg/mL 343.050

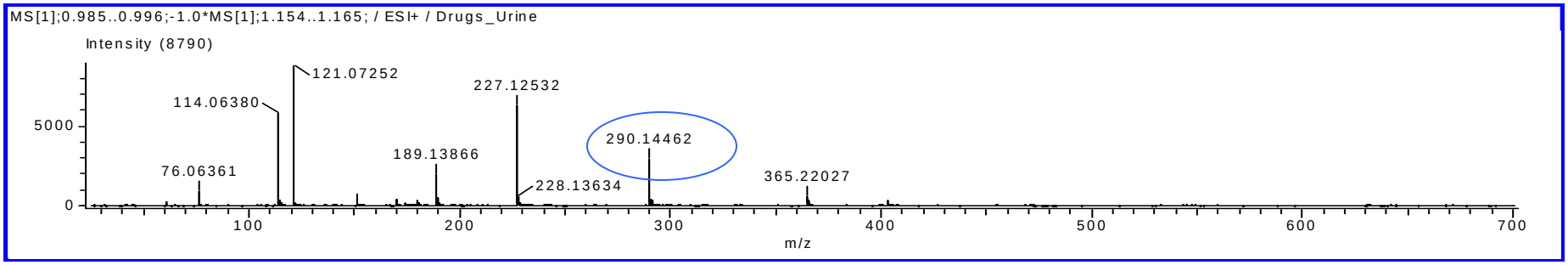


B-7.15. Triazolam 3.125 μg/mL 343.050

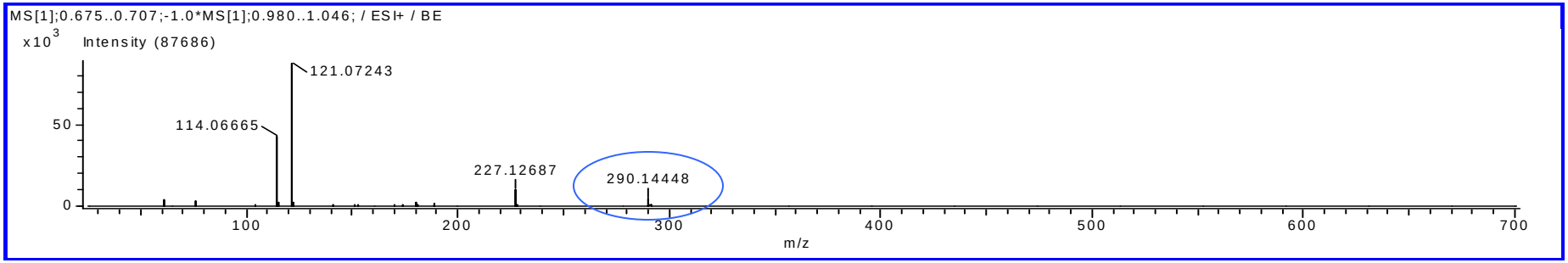


B-8. Cocaine and Metabolites

B-8.1. Benzoylecgonine 100 µg/mL 290.138

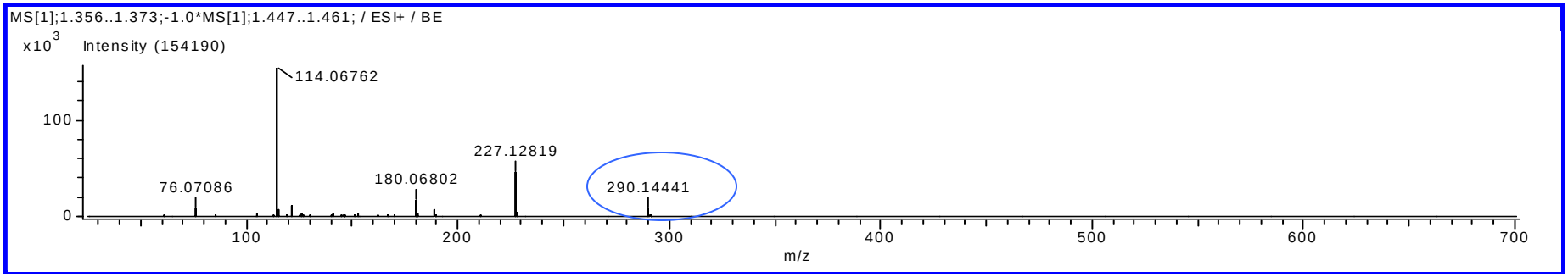


B-8.2. Benzoylecgonine 50 µg/mL 290.138

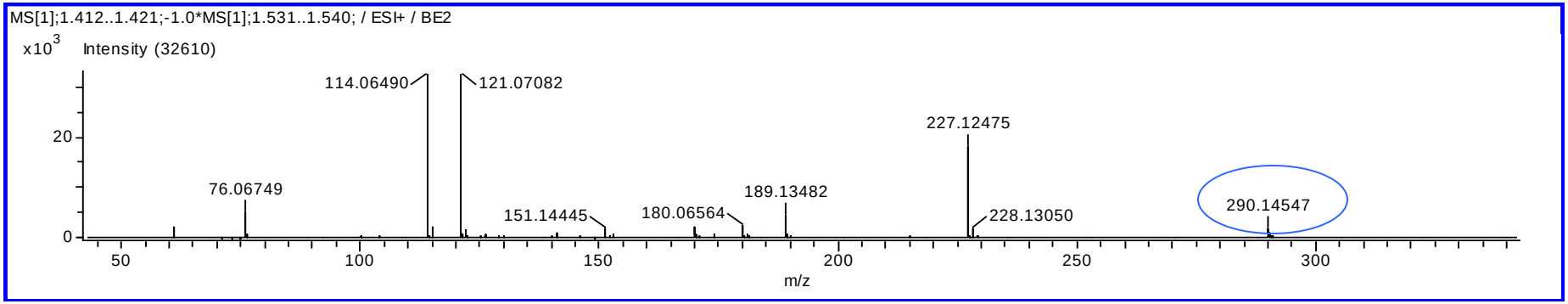


B-8. Cocaine and Metabolites (continued)

B-8.3. Benzoylecgonine 25 μg/mL 290.138

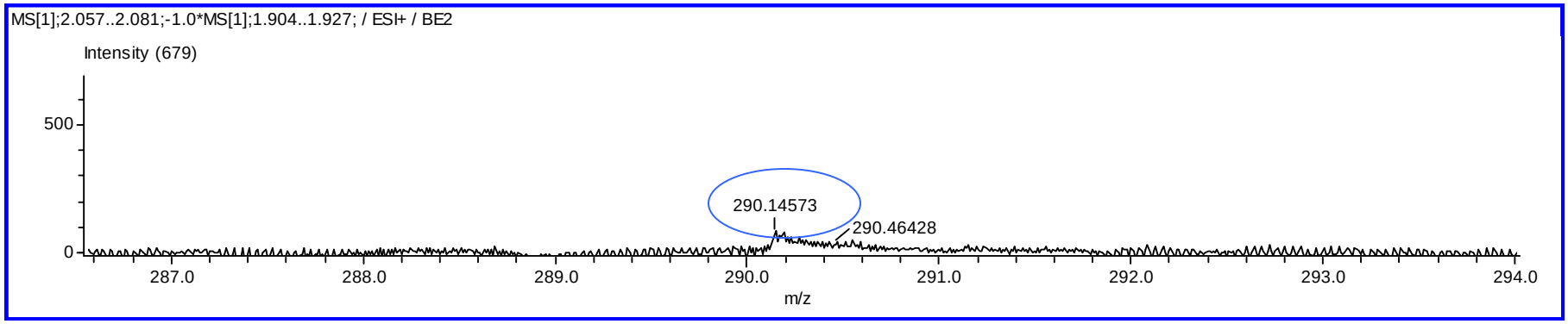


B-8.4. Benzoylecgonine 15 μg/mL 290.138



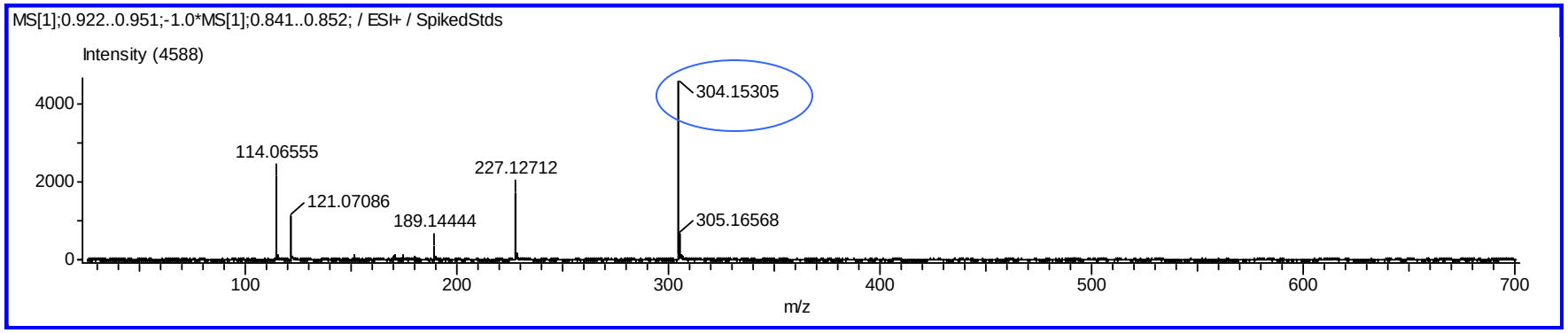
B-8. Cocaine and Metabolites (continued)

B-8.5. Benzoylecgonine 12.5 μg/mL 290.138

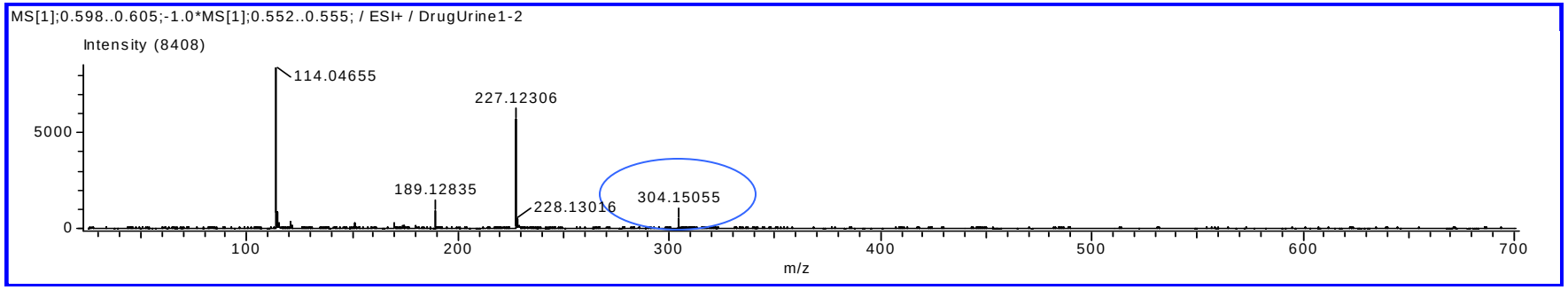


B-8. Cocaine and Metabolites (continued)

B-8.6. Cocaine 100 µg/mL 304.154

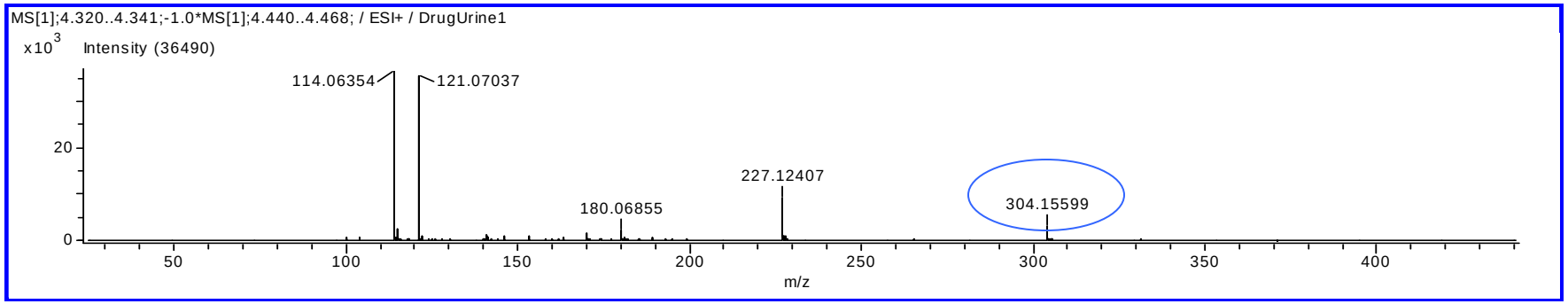


B-8.7. Cocaine 10 µg/mL 304.154

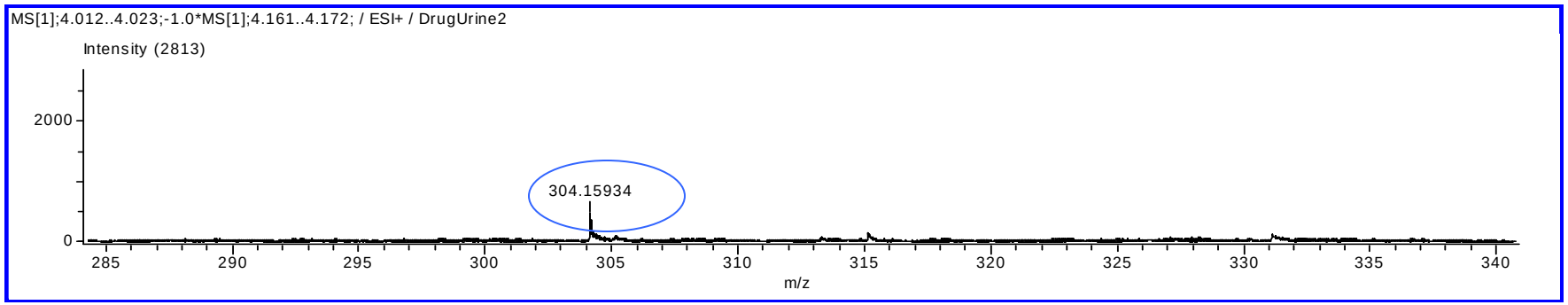


B-8. Cocaine and Metabolites (continued)

B-8.8. Cocaine 5 μg/mL 304.154

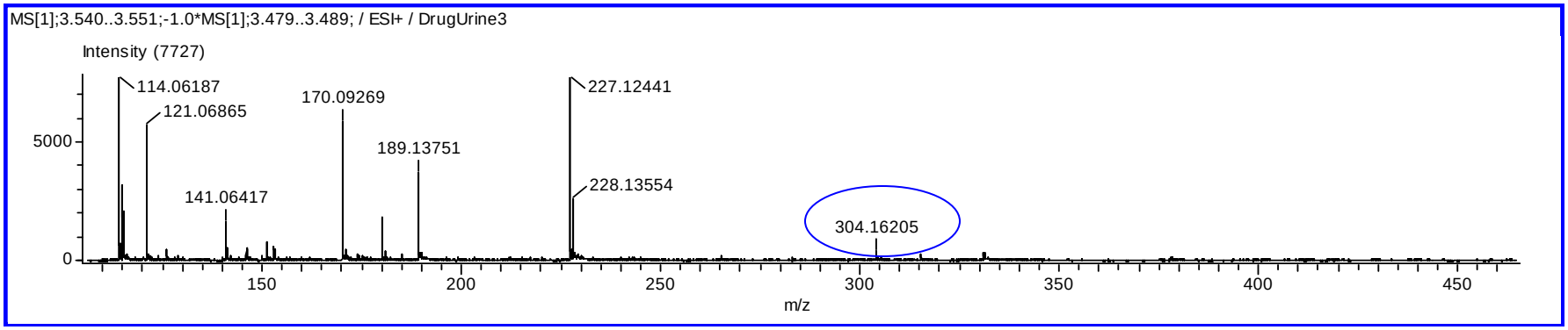


B-8.9. Cocaine 2.5 μg/mL 304.154

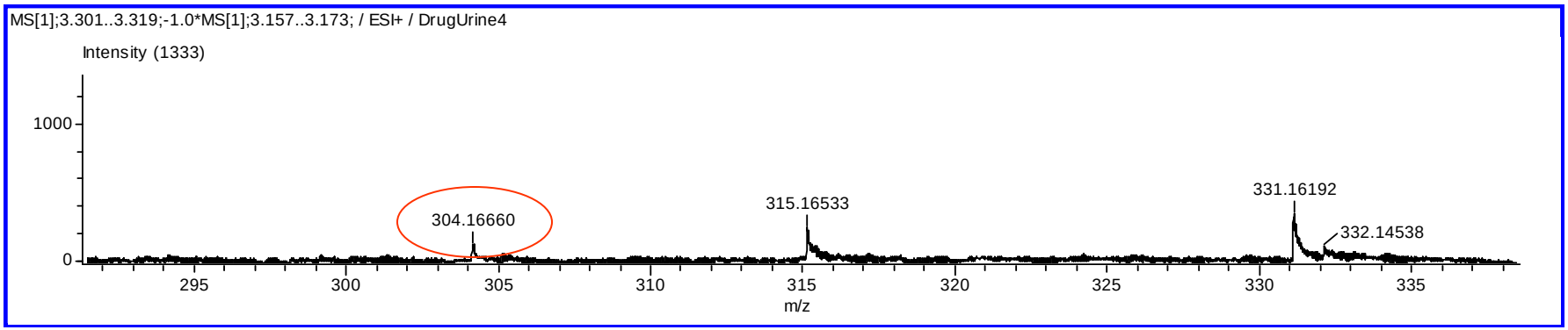


B-8. Cocaine and Metabolites (continued)

B-8.10. Cocaine 1.25 μg/mL 304.154

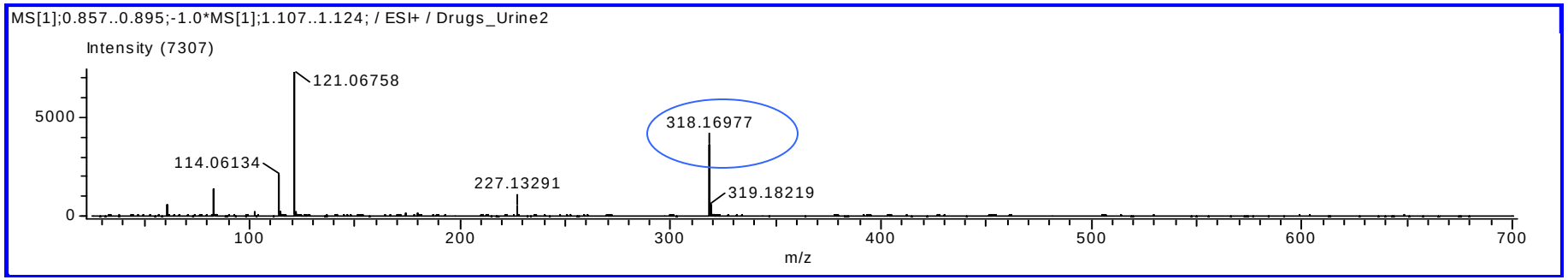


B-8.11. Cocaine 0.62 μg/mL 304.154

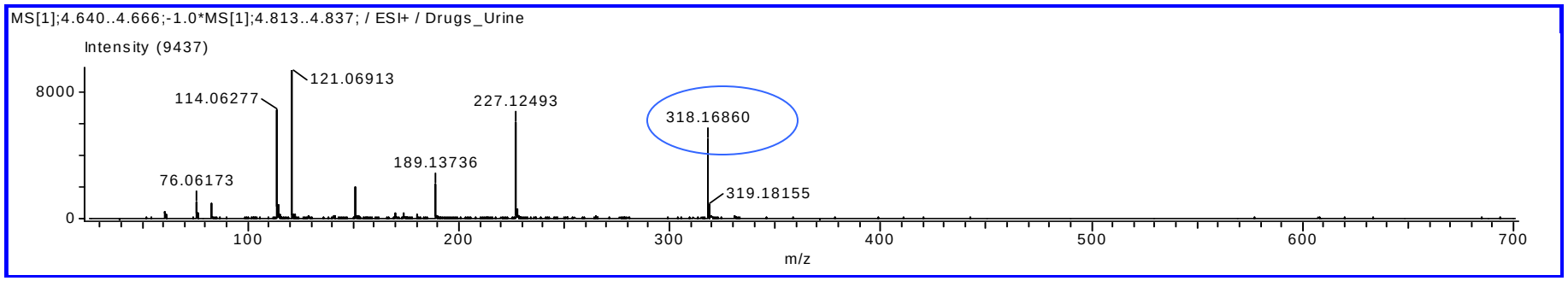


B-8. Cocaine and Metabolites (continued)

B-8.12. Cocaethylene 100 μg/mL 318.169

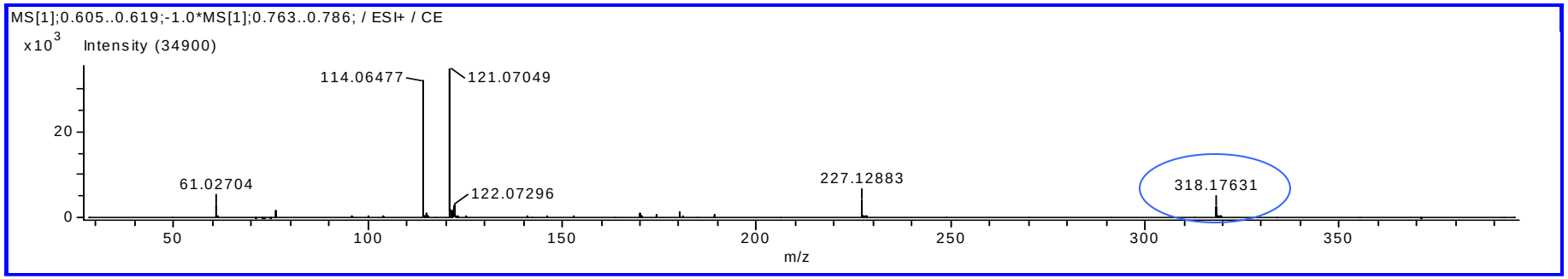


B-8.13. Cocaethylene 50 μg/mL 318.169

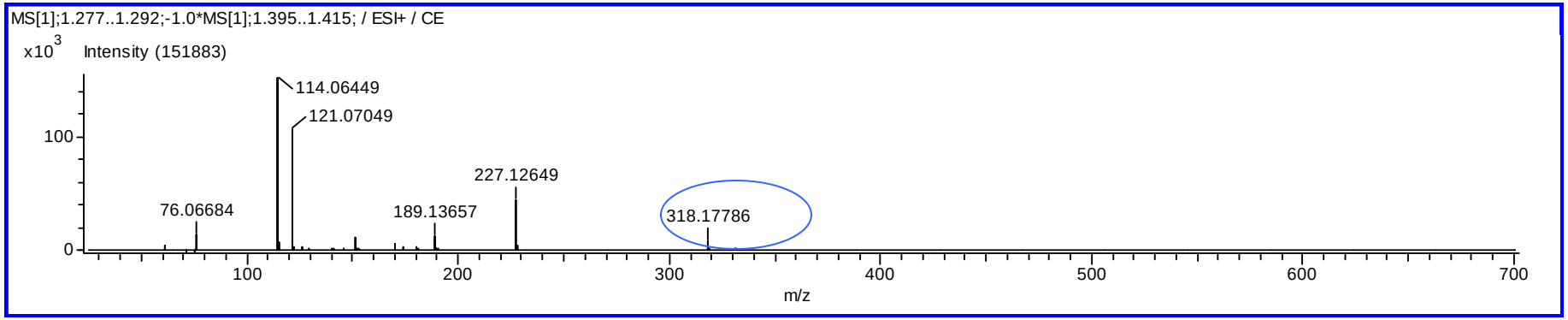


B-8. Cocaine and Metabolites (continued)

B-8.14. Cocaethylene 25 µg/mL 318.169

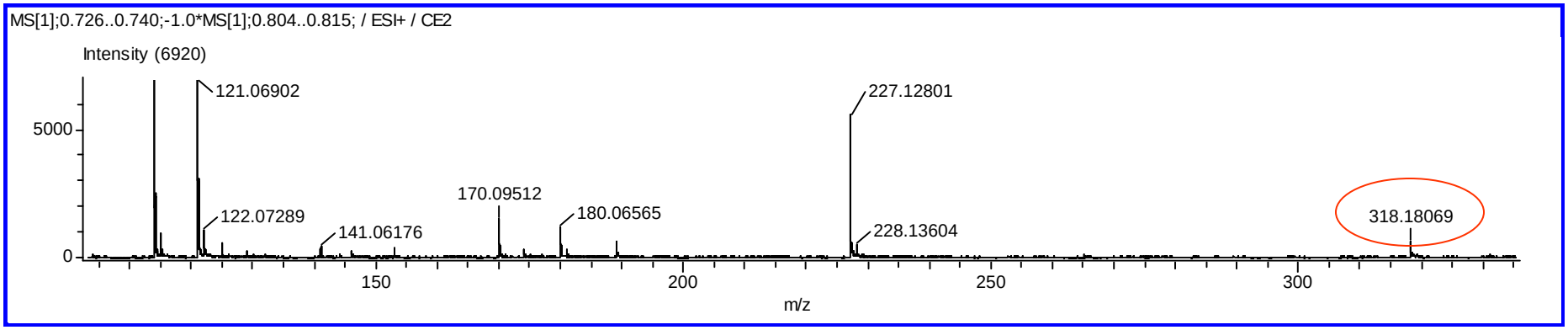


B-8.15. Cocaethylene 12.5 µg/mL 318.169

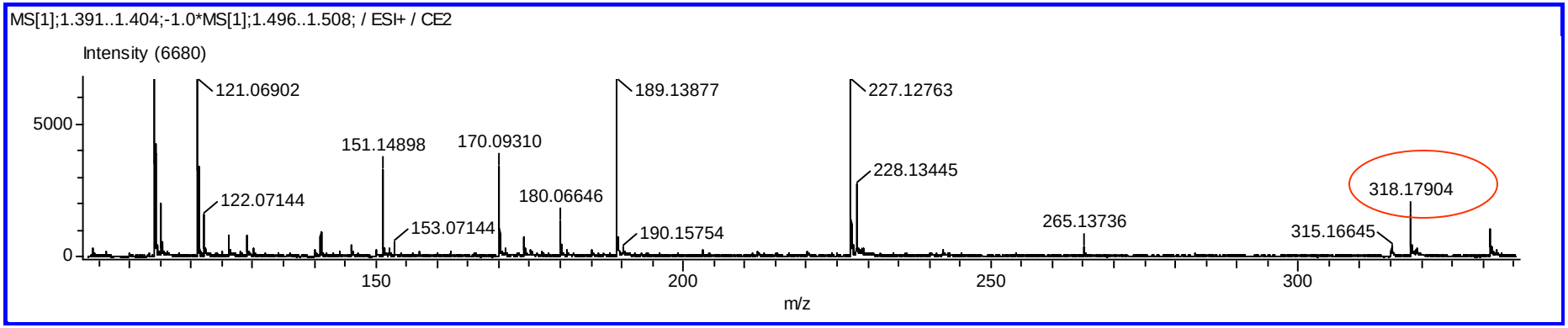


B-8. Cocaine and Metabolites (continued)

B-8.16. Cocaethylene 6.25 μg/mL 318.169

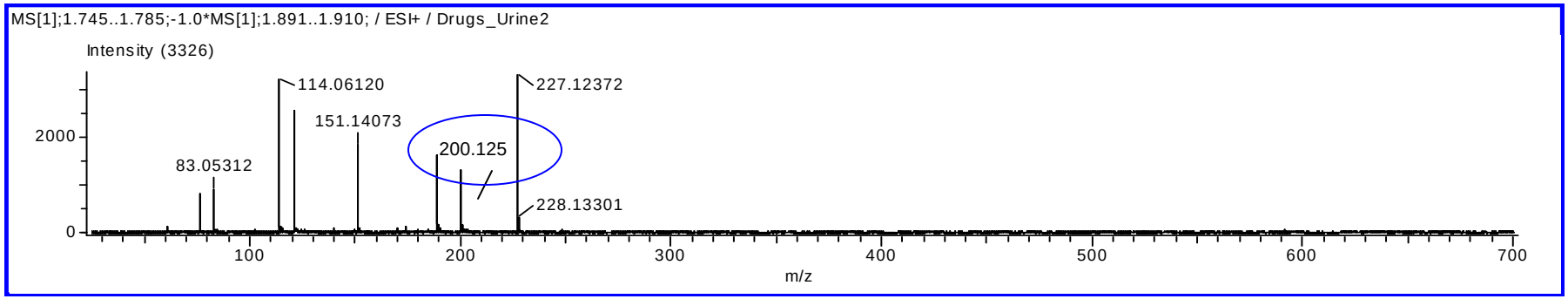


B-8.17. Cocaethylene 3.125 μg/mL 318.169

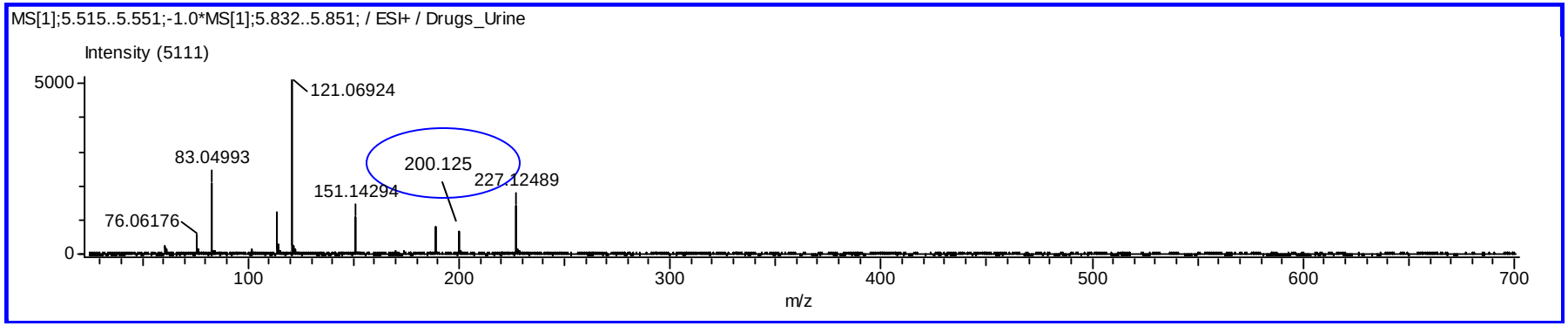


B-8. Cocaine and Metabolites (continued)

B-8.18. Ecgonine Methyl Ester 100 μg/mL 200.127

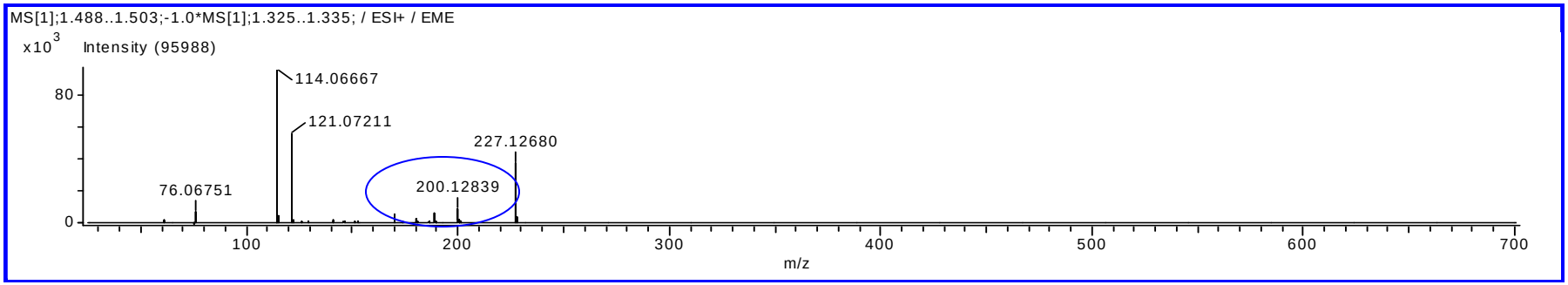


B-8.19. Ecgonine Methyl Ester 50 μg/mL 200.127

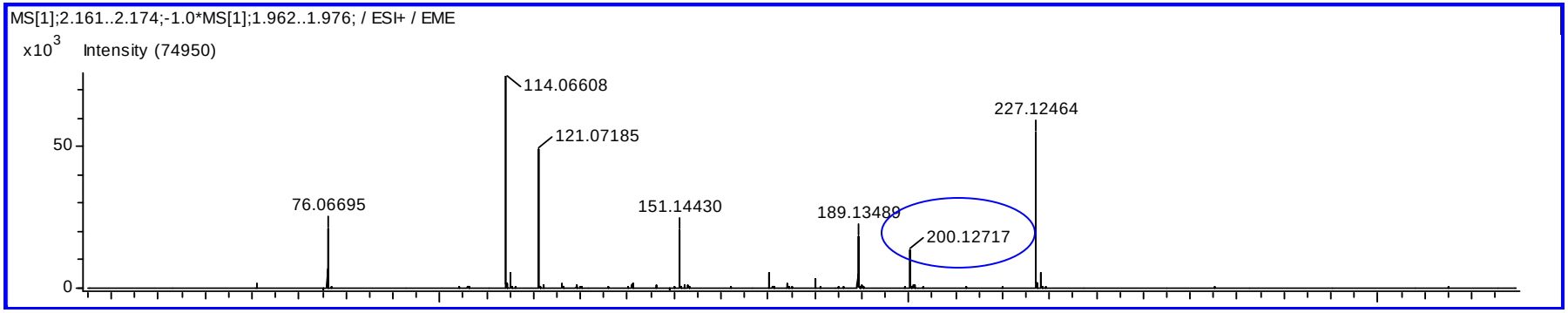


B-8. Cocaine and Metabolites (continued)

B-8.20. Ecgonine Methyl Ester 25 μg/mL 200.127

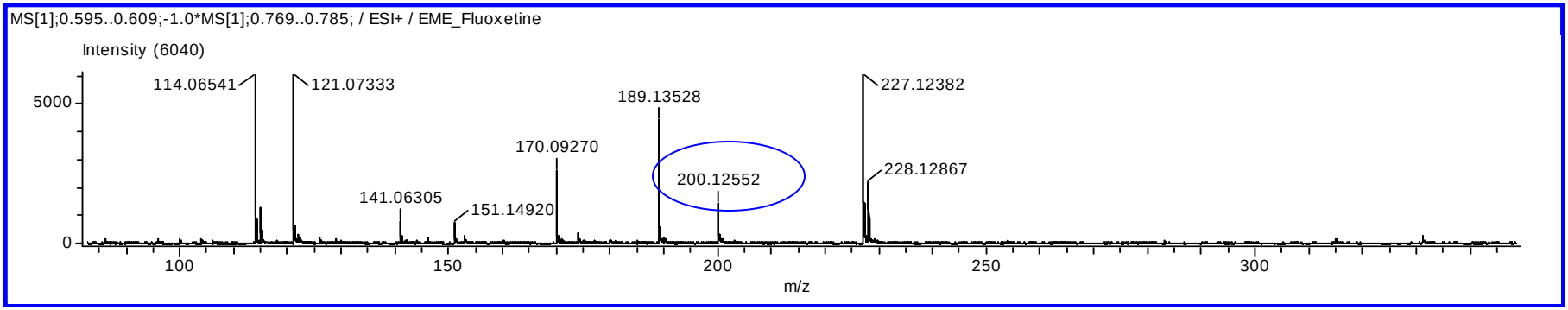


B-8.21. Ecgonine Methyl Ester 12.5 μg/mL 200.127

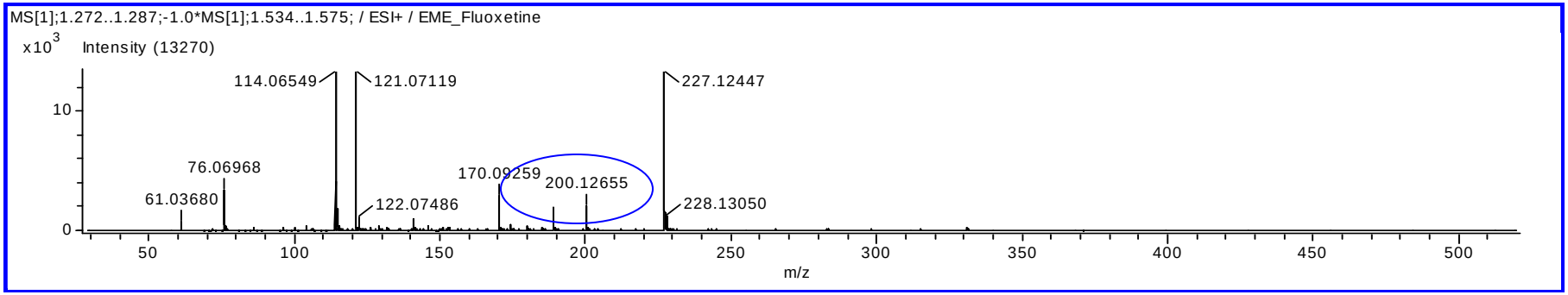


B-8. Cocaine and Metabolites (continued)

B-8.22. Ecgonine Methyl Ester 6.25 μg/mL 200.127

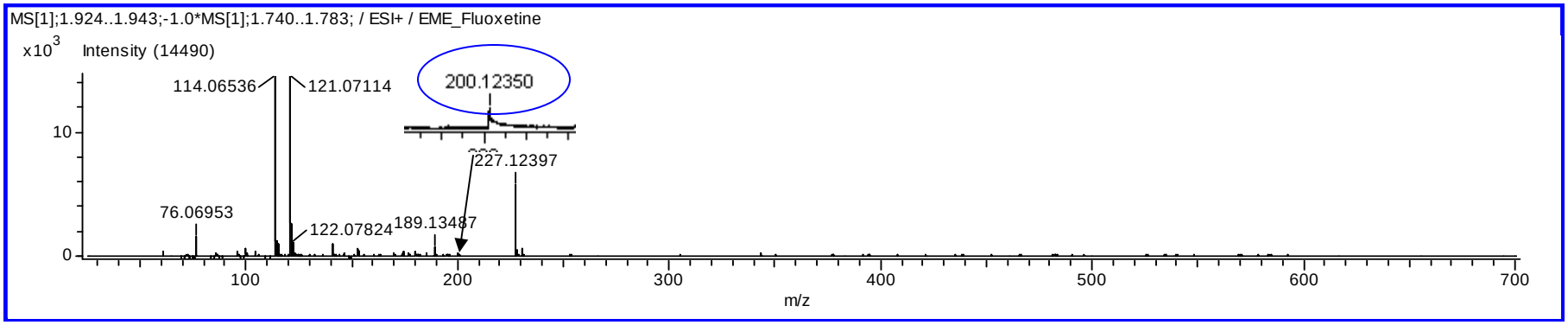


B-8.23. Ecgonine Methyl Ester 3.125 μg/mL 200.127



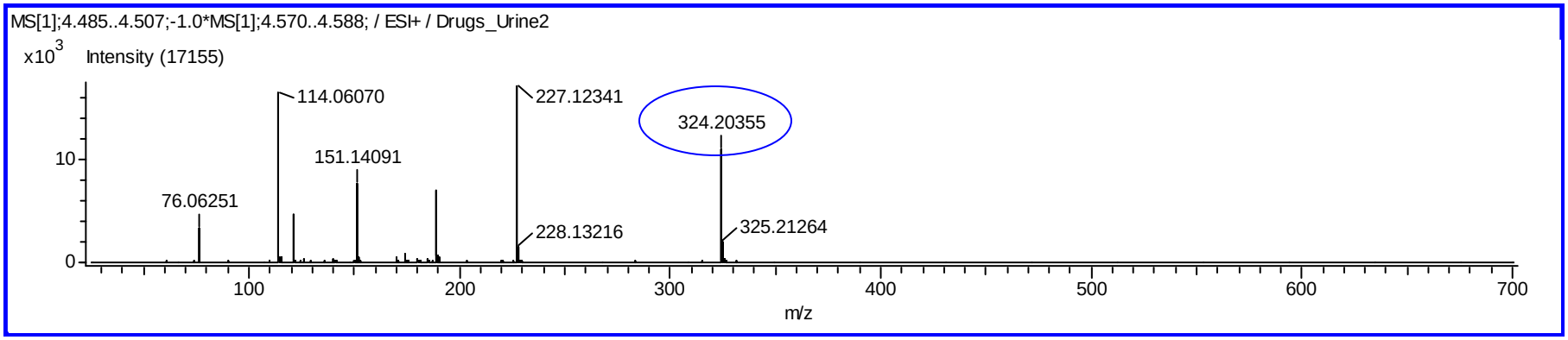
B-8. Cocaine and Metabolites (continued)

B-8.24. Ecgonine Methyl Ester 1.56 μg/mL 200.127

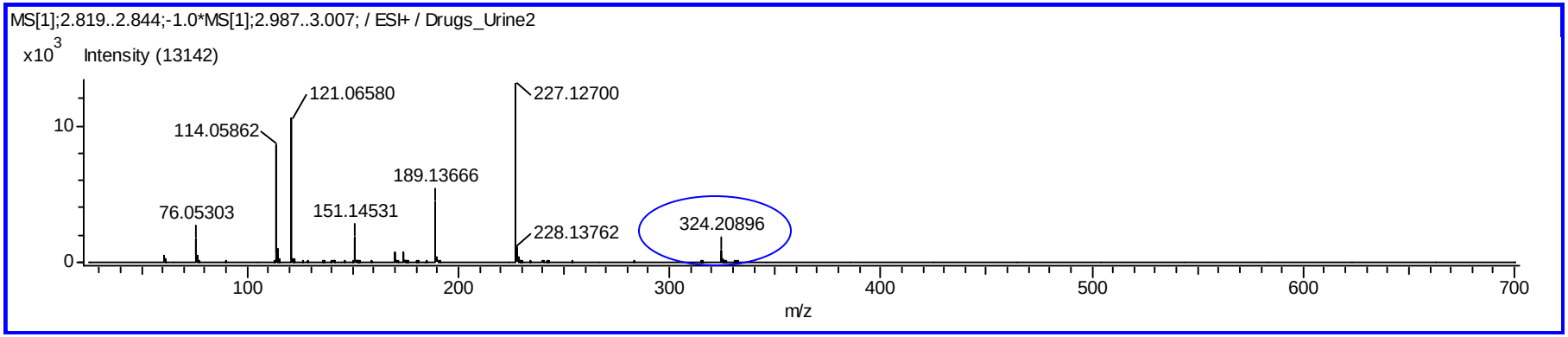


B-9. Hallucinogens

B-9.1. LSD 12.5 μg/mL 324.206

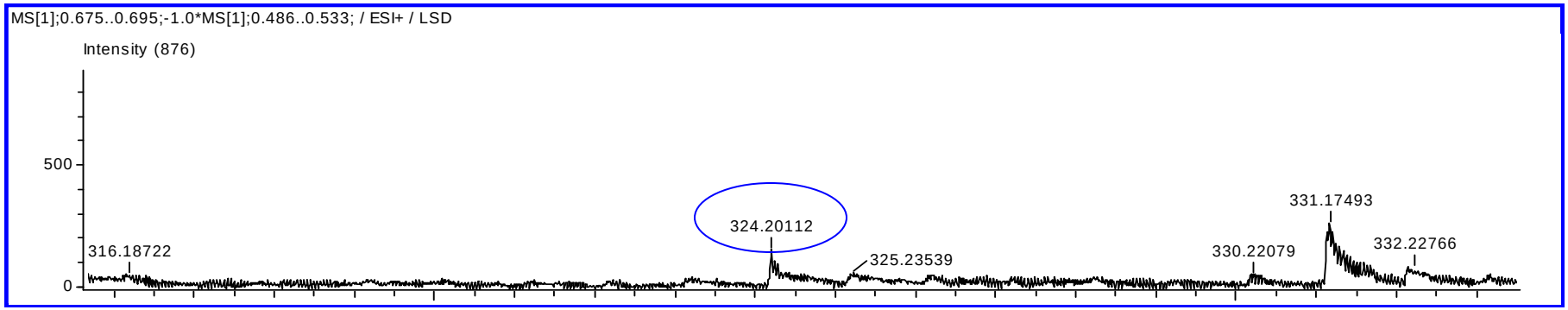


B-9.2. LSD 6.25 μg/mL 324.206



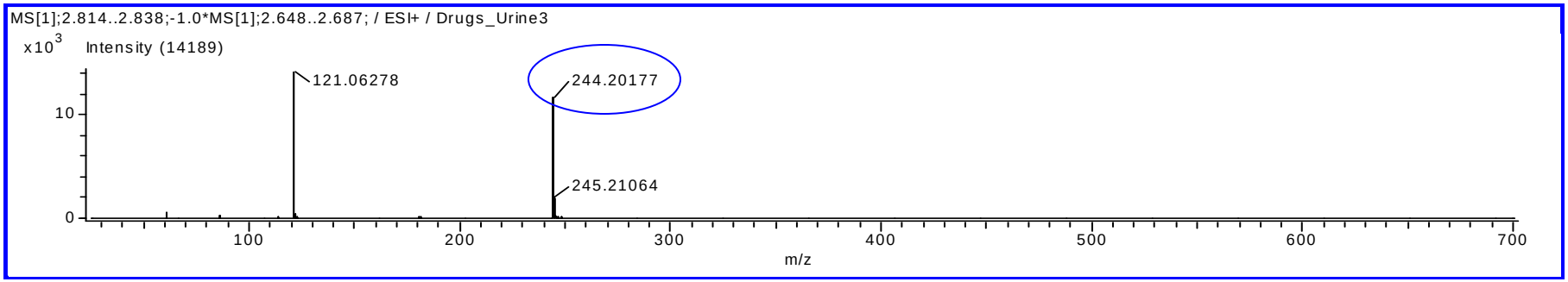
B-9. Hallucinogens (continued)

B-9.3. LSD 3.125 μg/mL 324.206

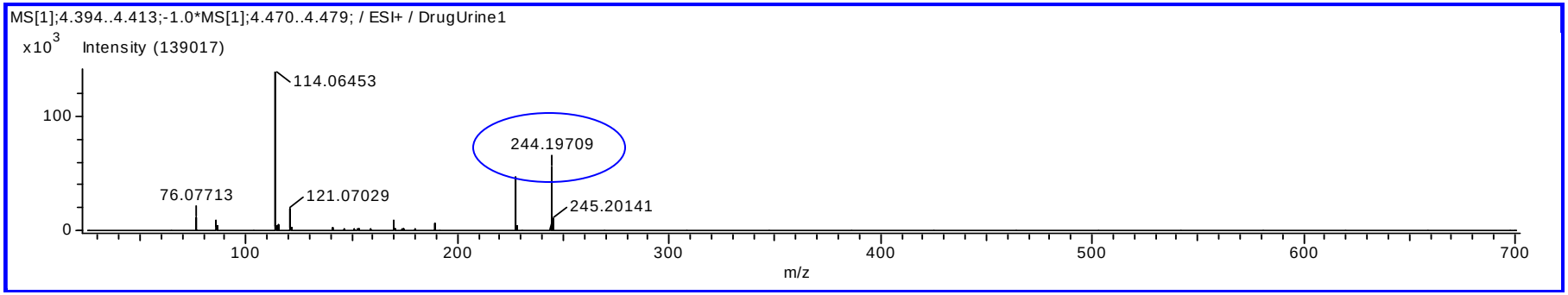


B-9. Hallucinogens (continued)

B-9.4. PCP 100 μg/mL 244.205

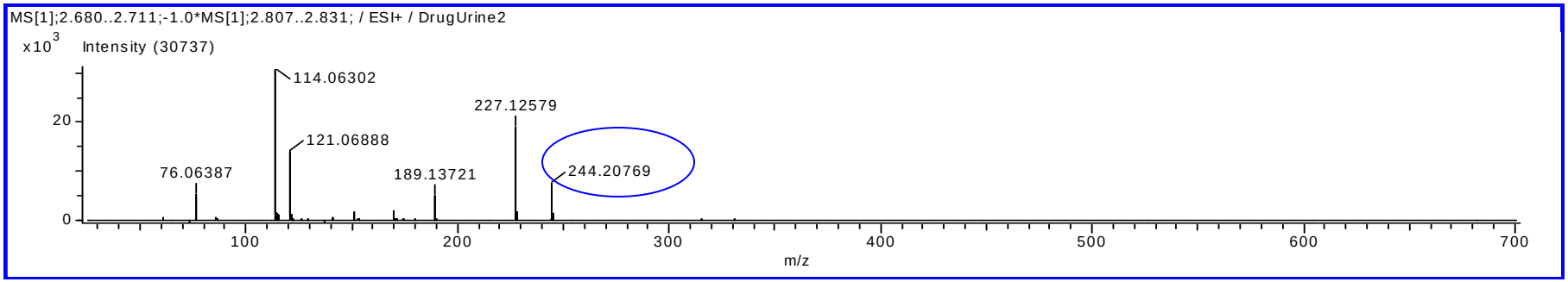


B-9.5. PCP 12.5 μg/mL 244.205

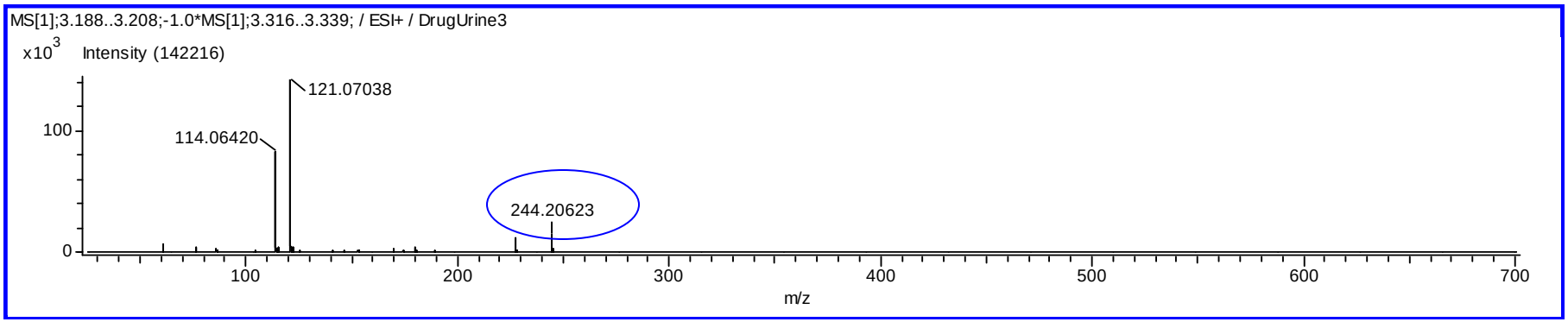


B-9. Hallucinogens (continued)

B-9.6. PCP 6.25 μg/mL 244.205

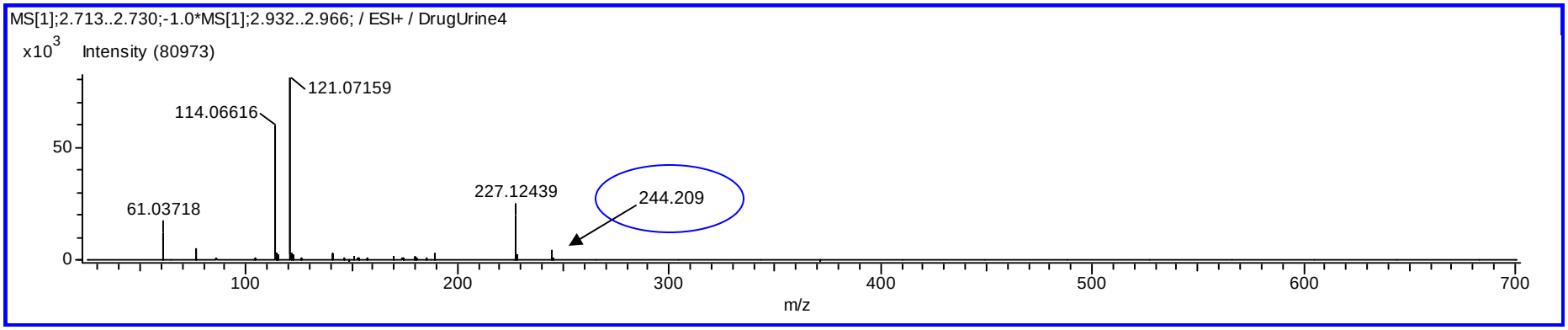


B-9.7. PCP 3.125 μg/mL 244.205

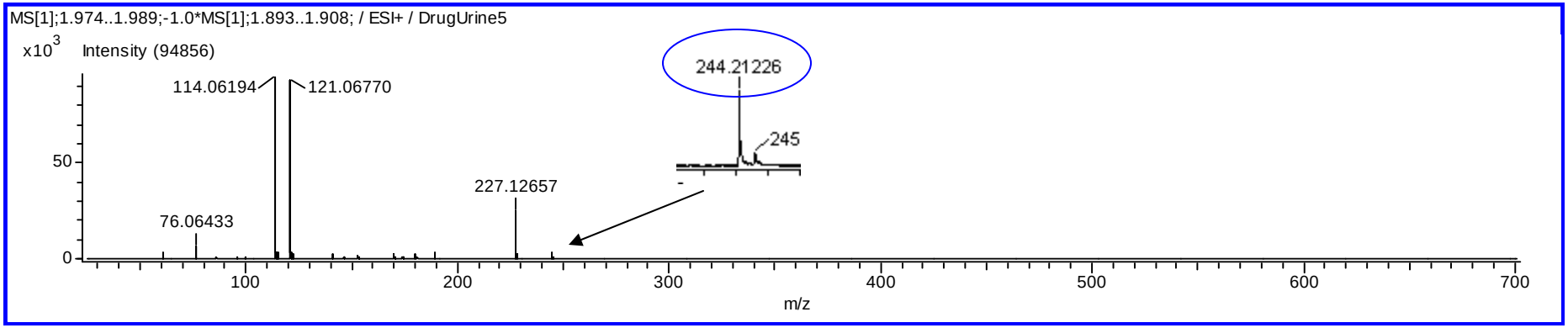


B-9. Hallucinogens (continued)

B-9.8. PCP 1.56 μg/mL 244.205

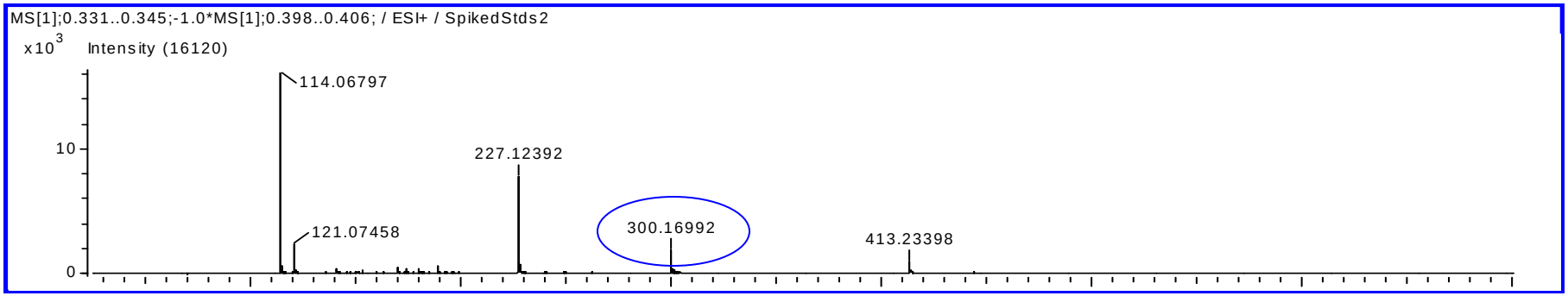


B-9.9. PCP 0.78 μg/mL 244.205

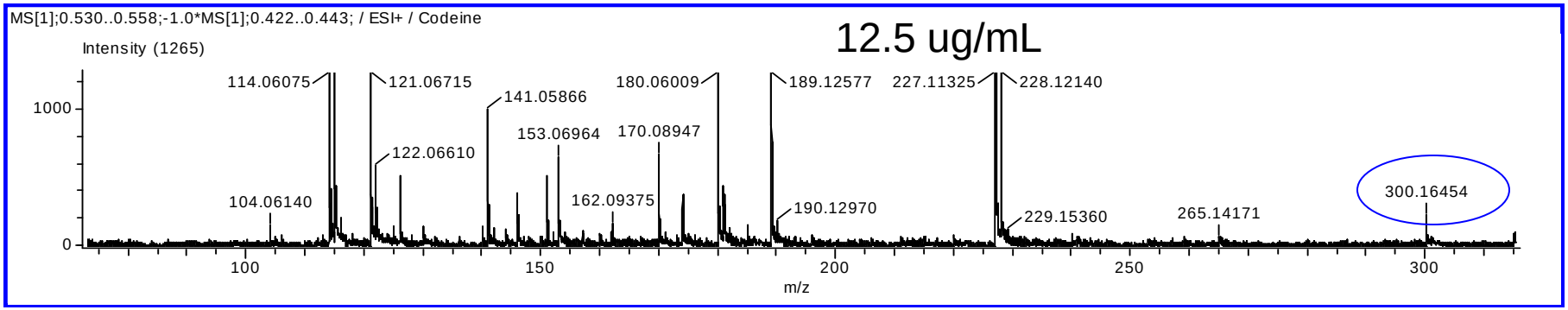


B-10. Opiates

B-10.1. Codeine 100 μg/mL 300.159

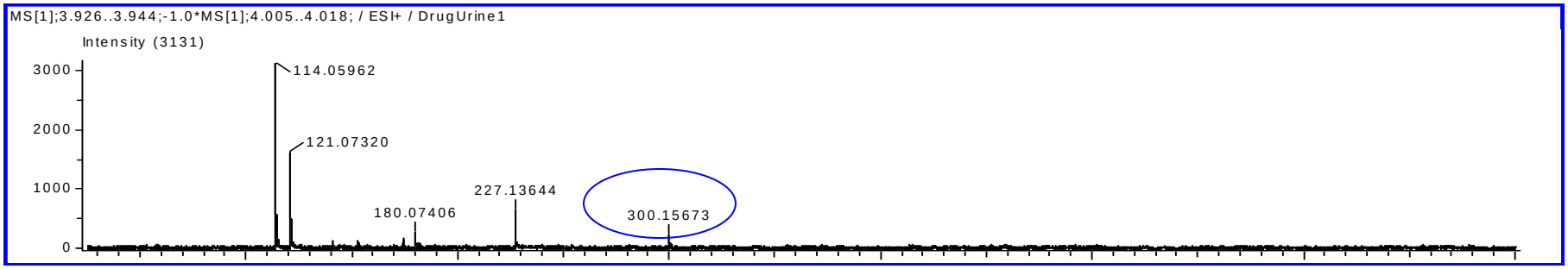


B-10.2. Codeine 12.5 μg/mL 300.159

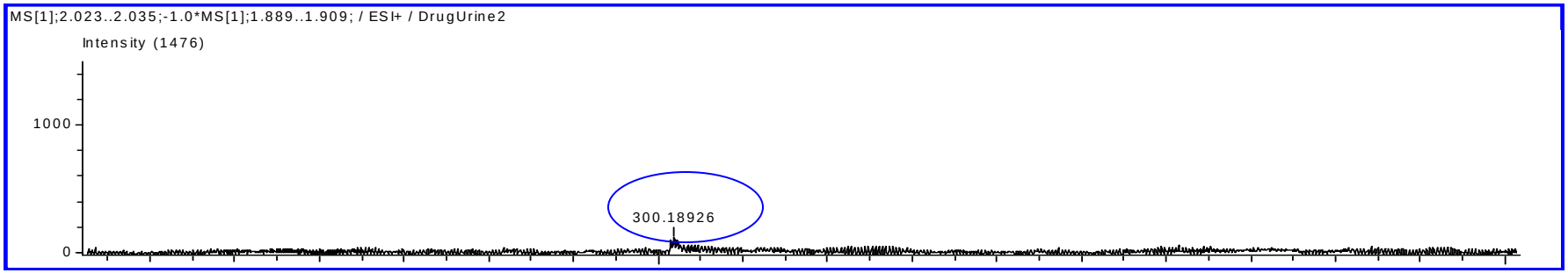


B-10. Opiates (continued)

B-10.3. Codeine 10 μg/mL 300.159

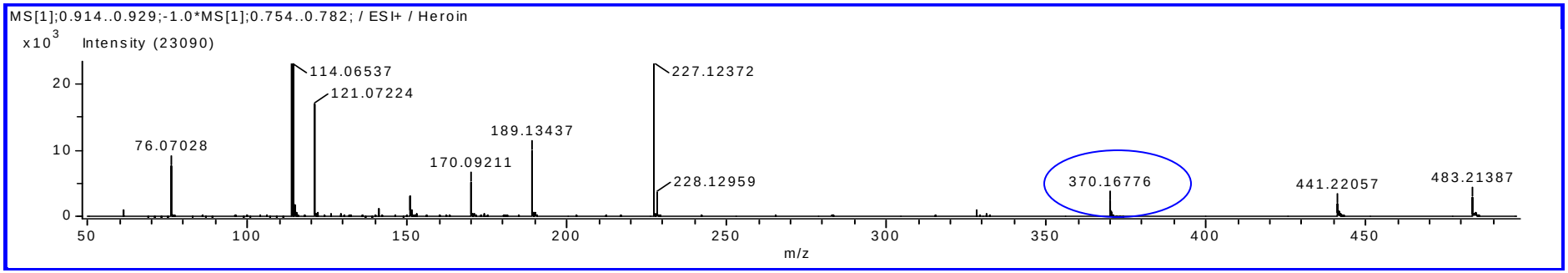


B-10.4. Codeine 6.25 μg/mL 300.159

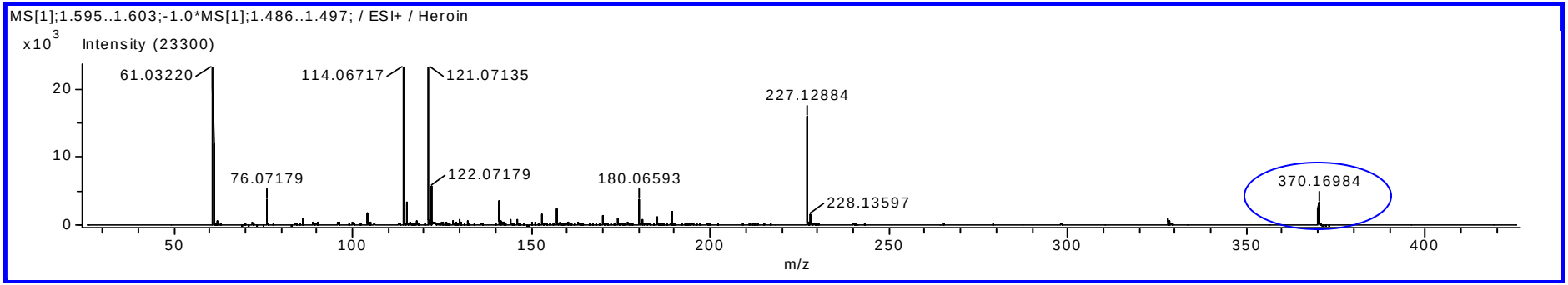


B-10. Opiates (continued)

B-10.5. Heroin 100 μg/mL 370.164

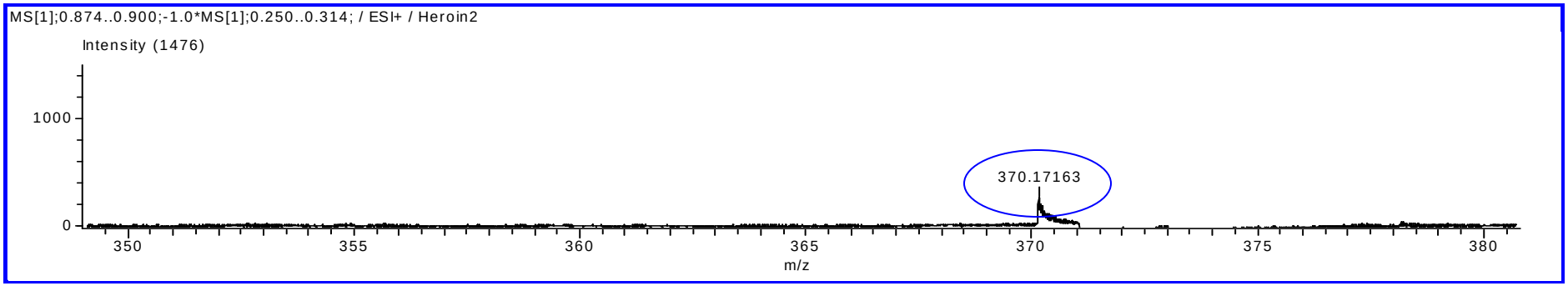


B-10.6. Heroin 50 μg/mL 370.164



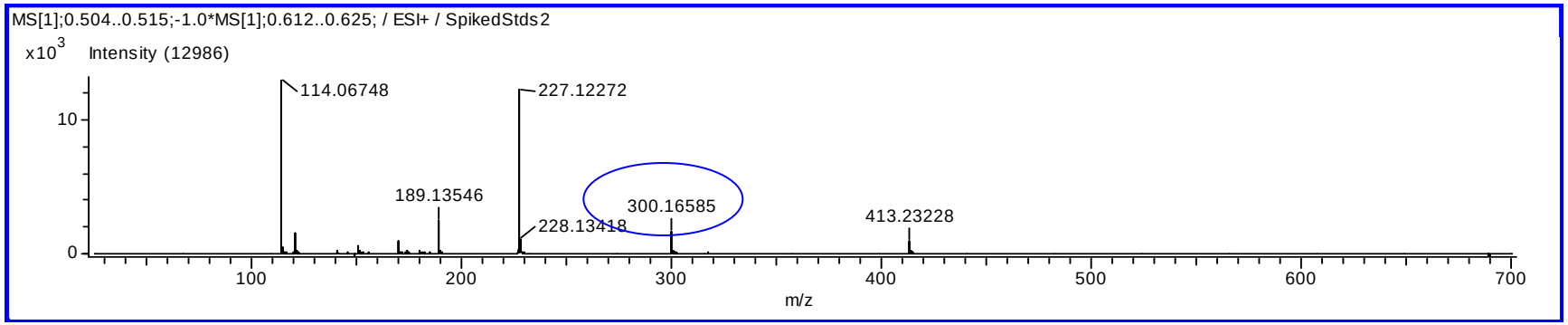
B-10. Opiates (continued)

B-10.7. Heroin 25 μg/mL 370.164

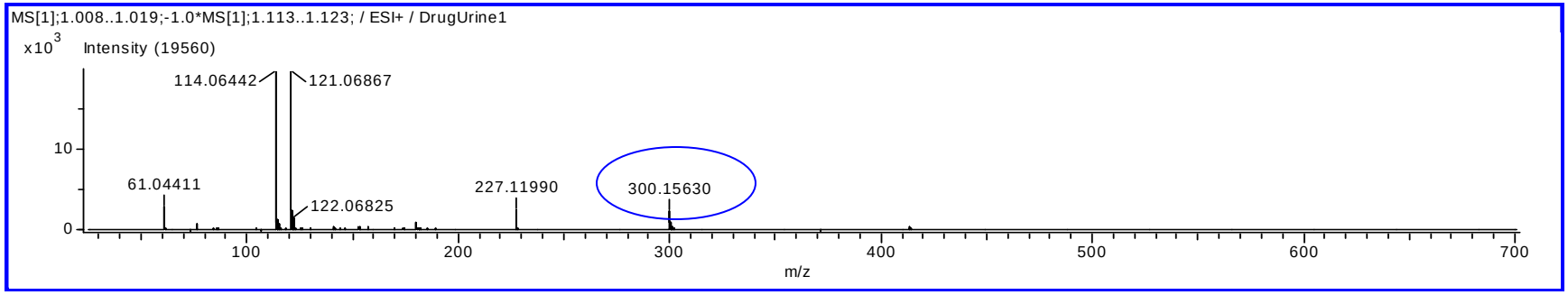


B-10. Opiates (continued)

B-10.8. Hydrocodone 100 μg/mL 300.159

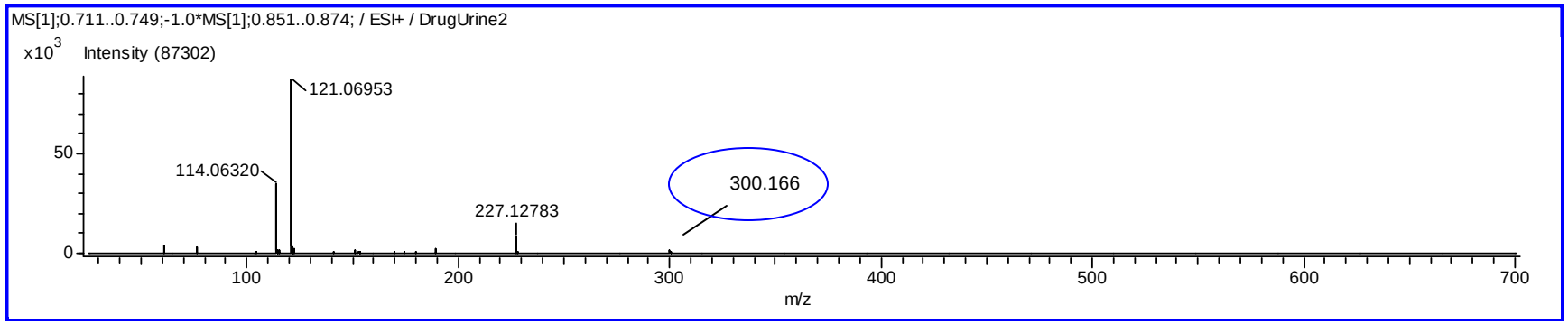


B-10.9. Hydrocodone 12.5 μg/mL 300.159

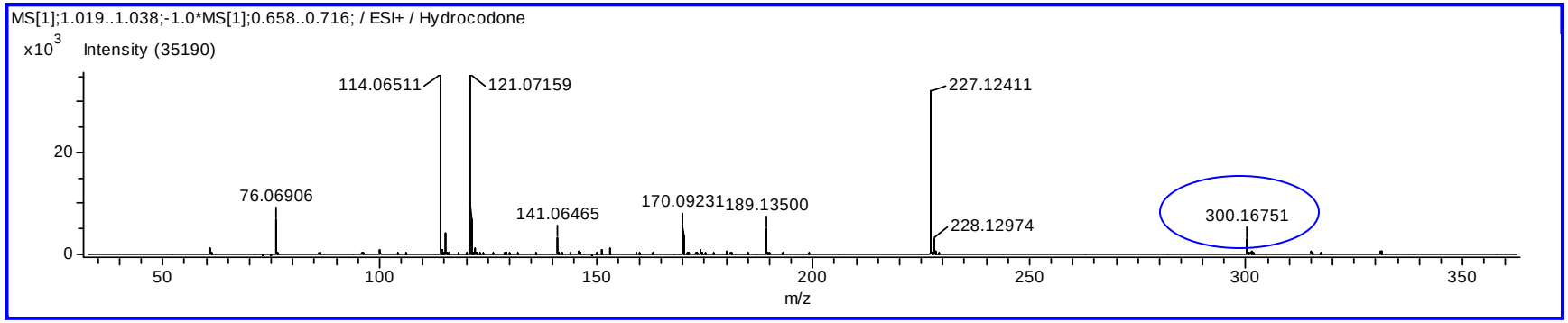


B-10. Opiates (continued)

B-10.10. Hydrocodone 6.25 μg/mL 300.159

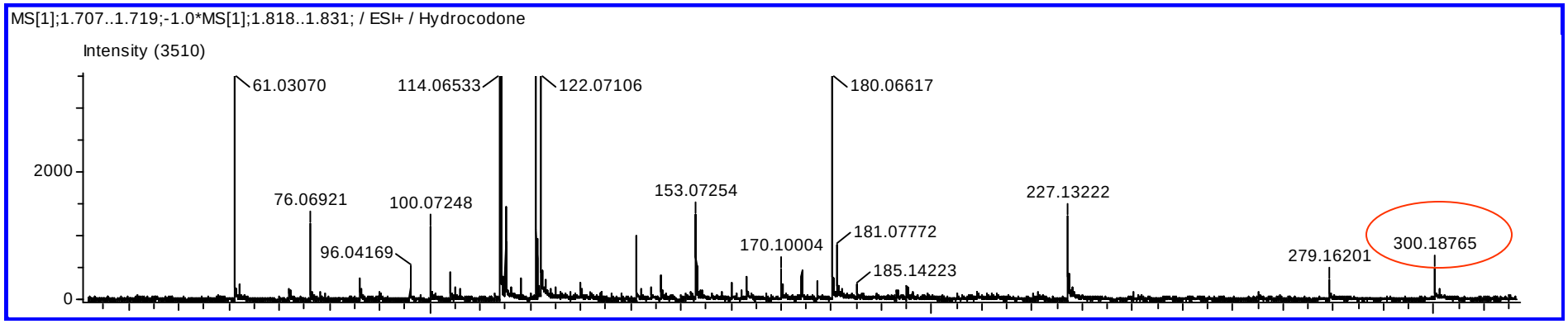


B-10.11. Hydrocodone 3.125 μg/mL 300.159



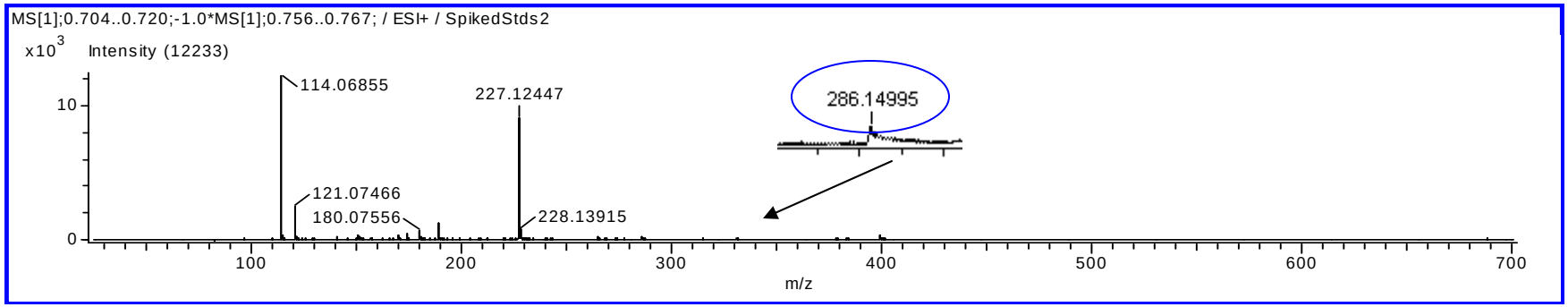
B-10. Opiates (continued)

B-10.12. Hydrocodone 1.56 μg/mL 300.159

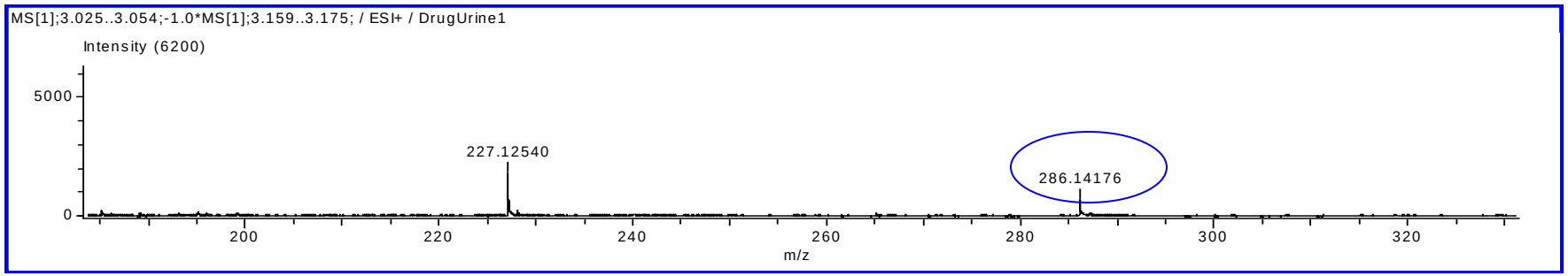


B-10. Opiates (continued)

B-10.13. Morphine 100 µg/mL 286.143

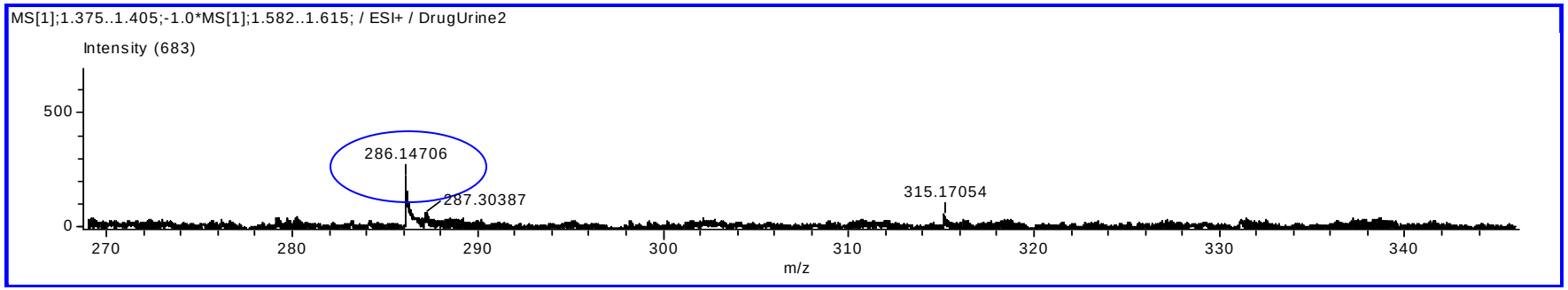


B-10.14. Morphine 12.5 µg/mL 286.143

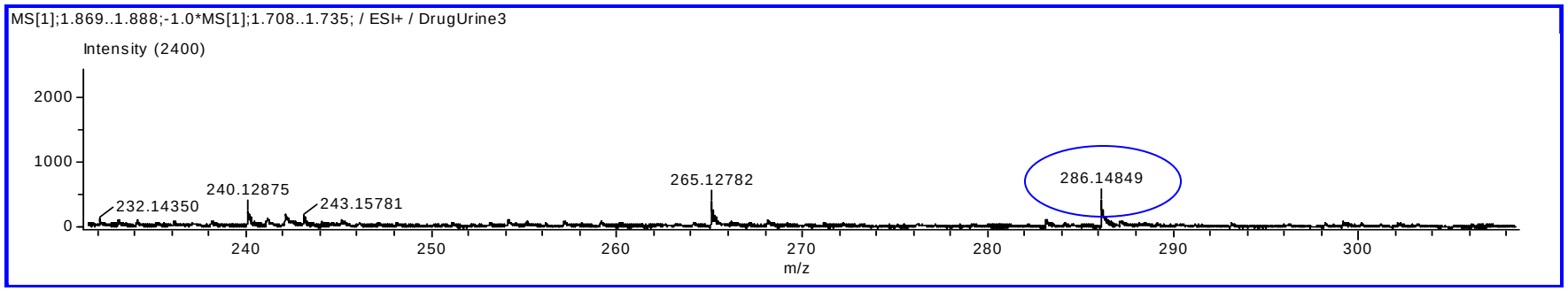


B-10. Opiates (continued)

B-10.15. Morphine 6.25 μg/mL 286.143

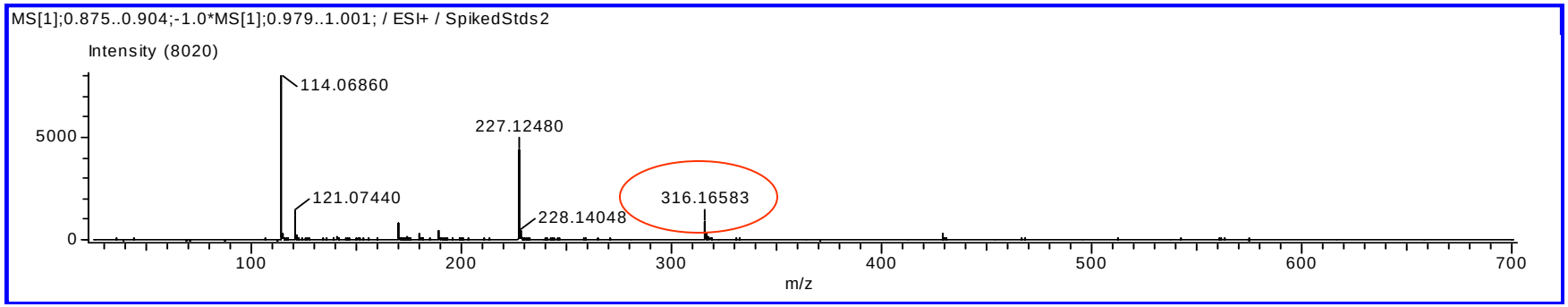


B-10.16. Morphine 3.125 μg/mL 286.143

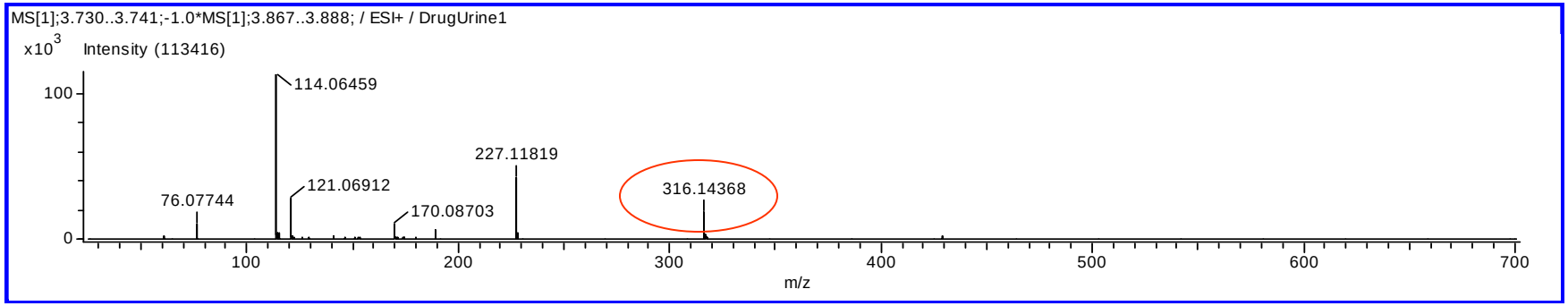


B-10. Opiates (continued)

B-10.17. Oxycodone 100 μg/mL 316.154

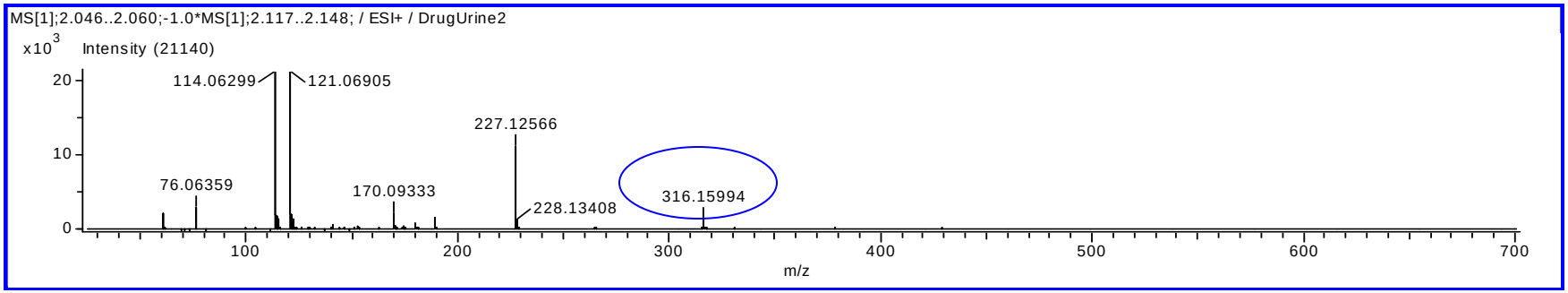


B-10.18. Oxycodone 12.5 μg/mL 316.154

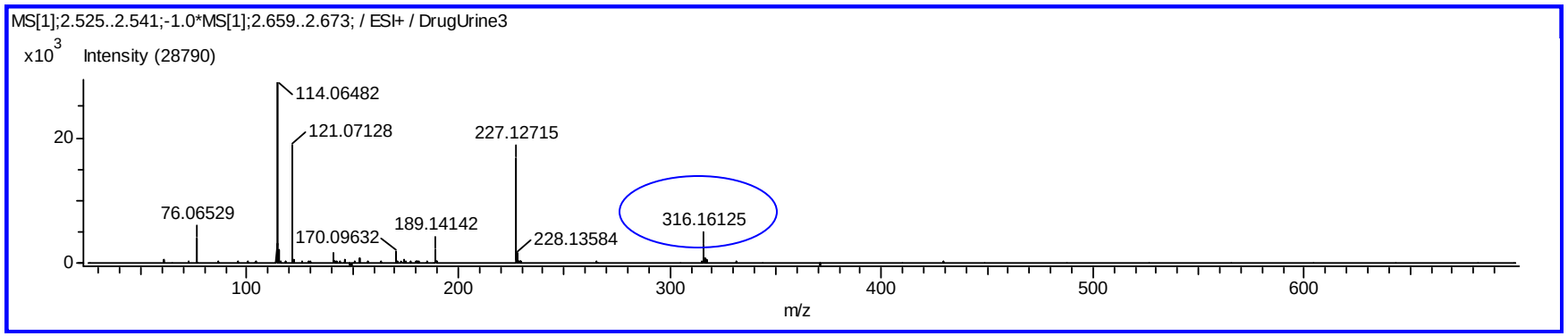


B-10. Opiates (continued)

B-10.19. Oxycodone 6.25 μg/mL 316.154

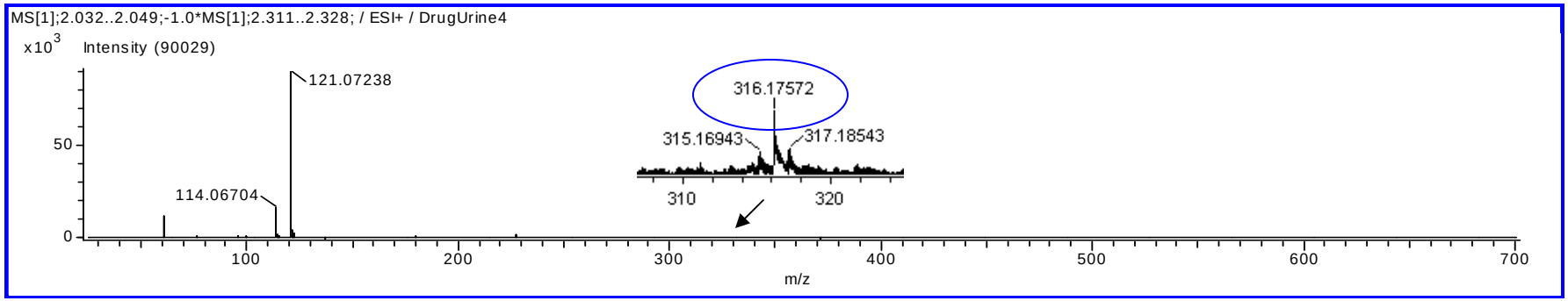


B-10.20. Oxycodone 3.125 μg/mL 316.154

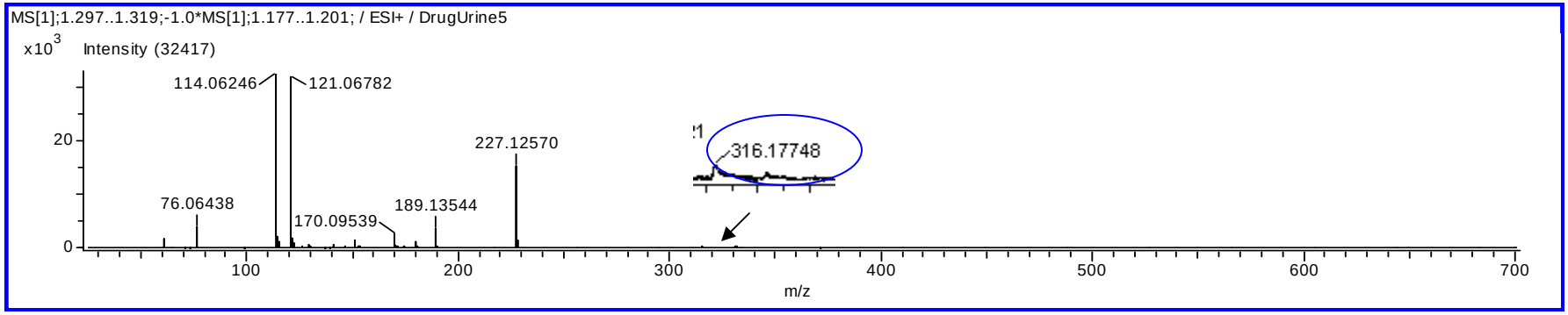


B-10. Opiates (continued)

B-10.21. Oxycodone 1.56 µg/mL 316.154

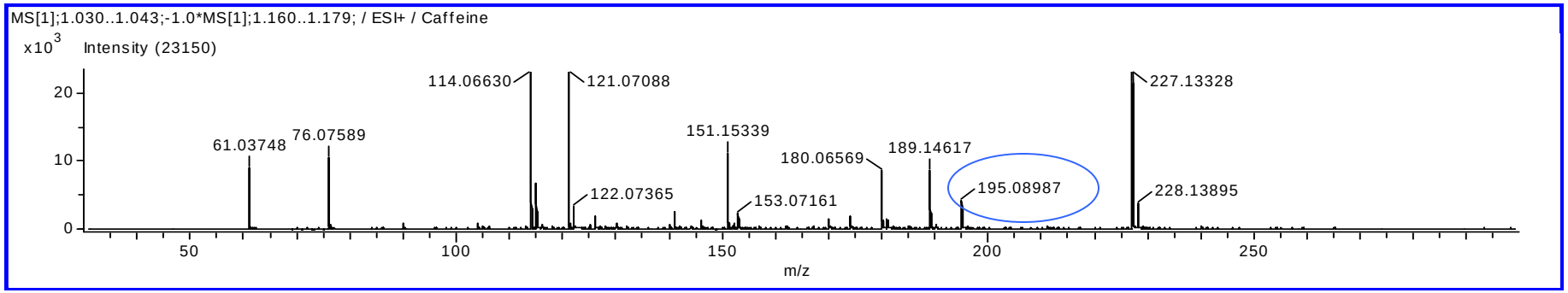


B-10.22. Oxycodone 0.78 µg/mL 316.154

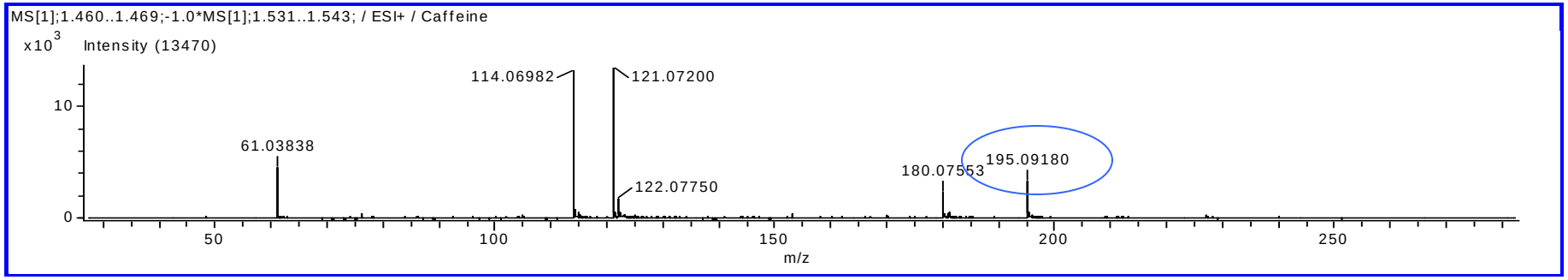


B-11. Miscellaneous

B-11.1. Caffeine 100 µg/mL 195.087

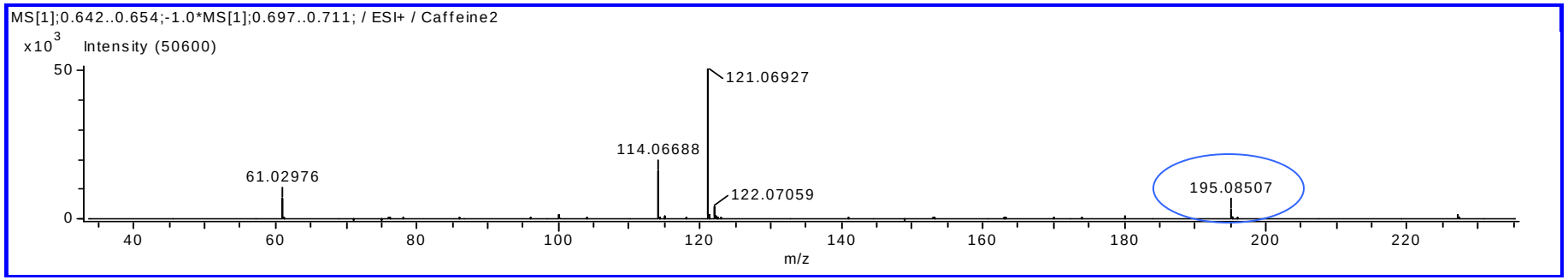


B-11.2. Caffeine 50 µg/mL 195.087

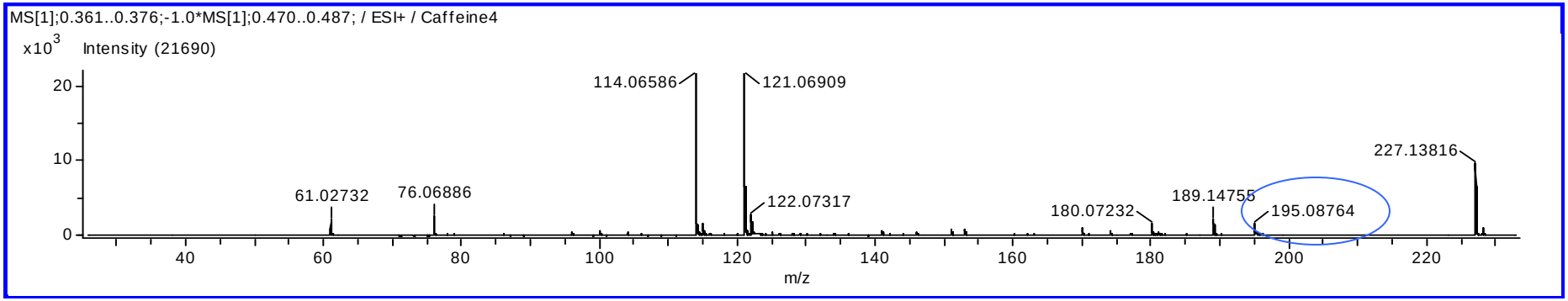


B-11. Miscellaneous (continued)

B-11.3. Caffeine 25 μg/mL 195.087

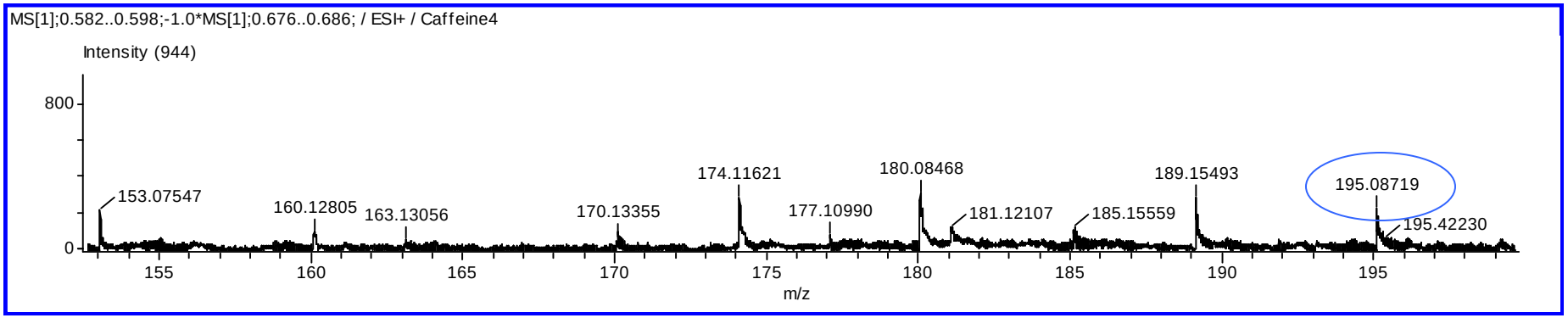


B-11.4. Caffeine 20 μg/mL 195.087



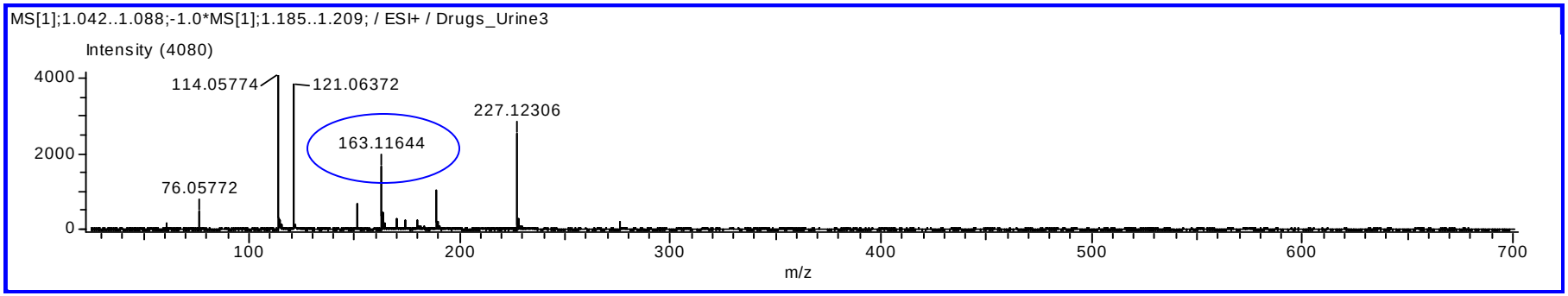
B-11. Miscellaneous (continued)

B-11.5. Caffeine 15 μg/mL 195.087

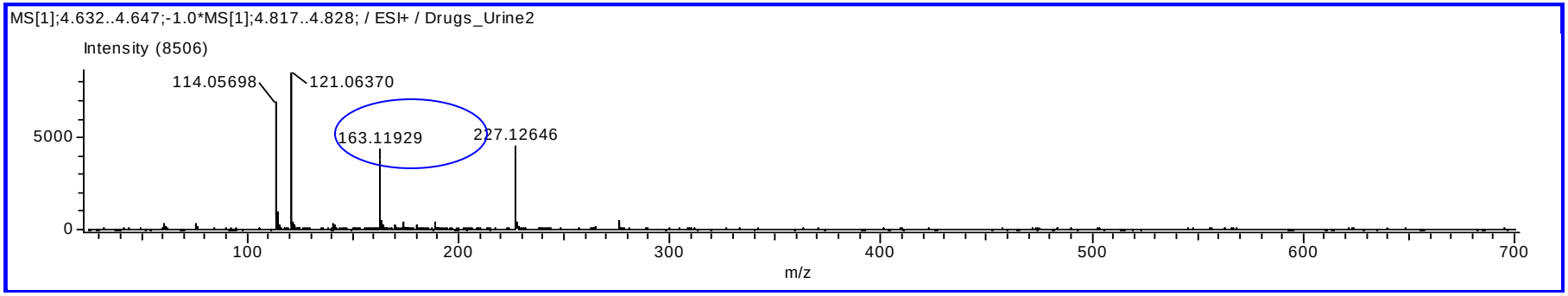


B-11. Miscellaneous (continued)

B-11.6. Nicotine 100 μg/mL 163.122

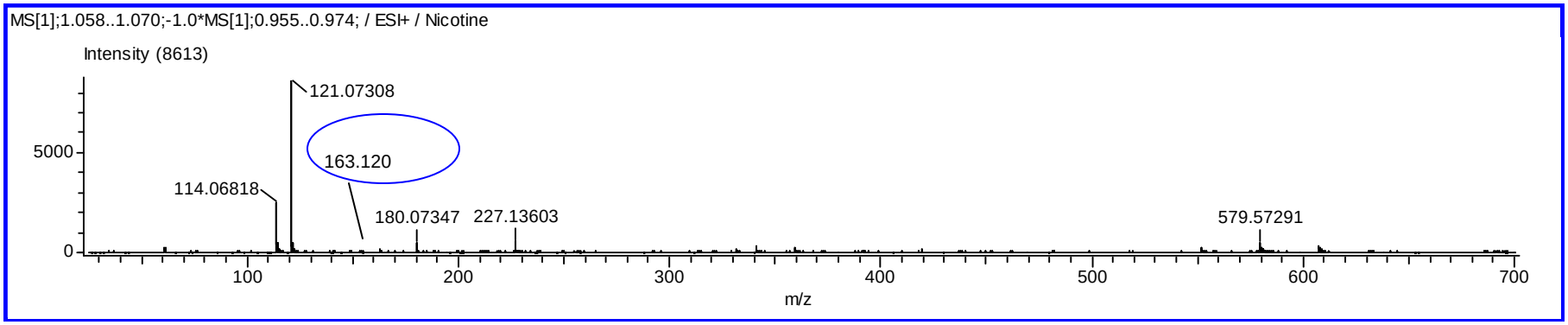


B-11.7. Nicotine 50 μg/mL 163.122

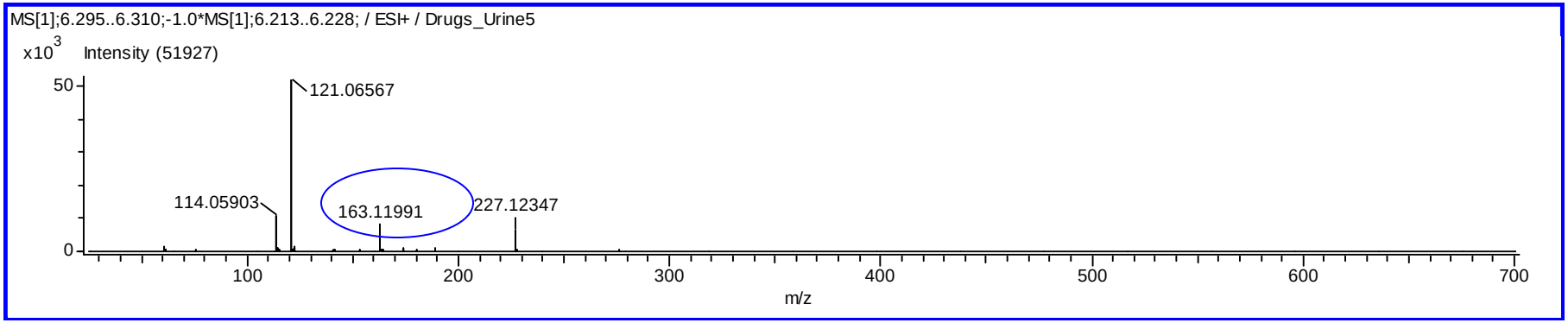


B-11. Miscellaneous (continued)

B-11.8. Nicotine 25 μg/mL 163.122

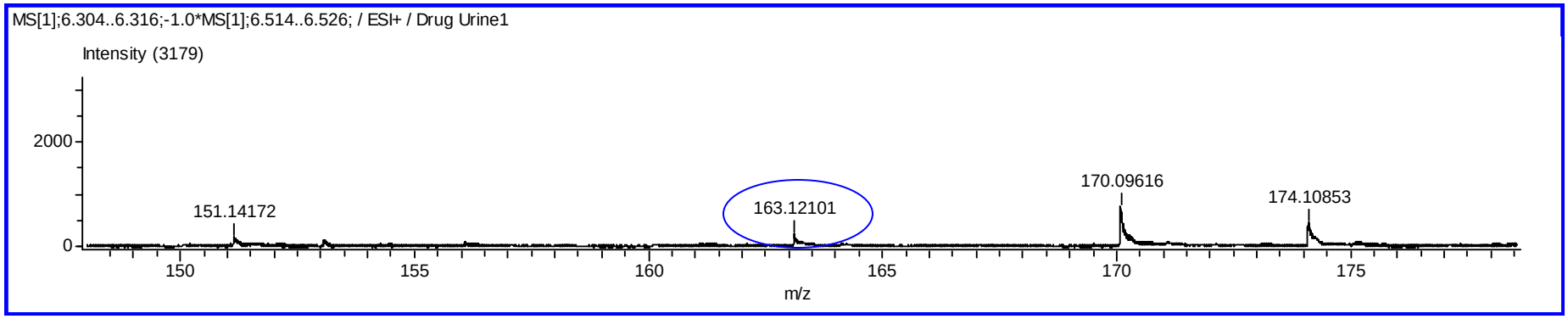


B-11.9. Nicotine 12.5 μg/mL 163.122

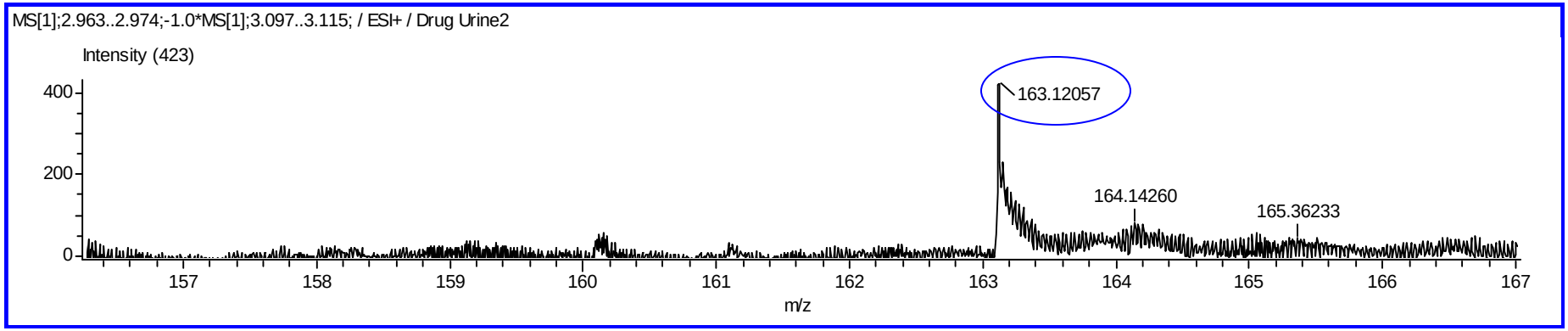


B-11. Miscellaneous (continued)

B-11.10. Nicotine 6.25 μg/mL 163.122

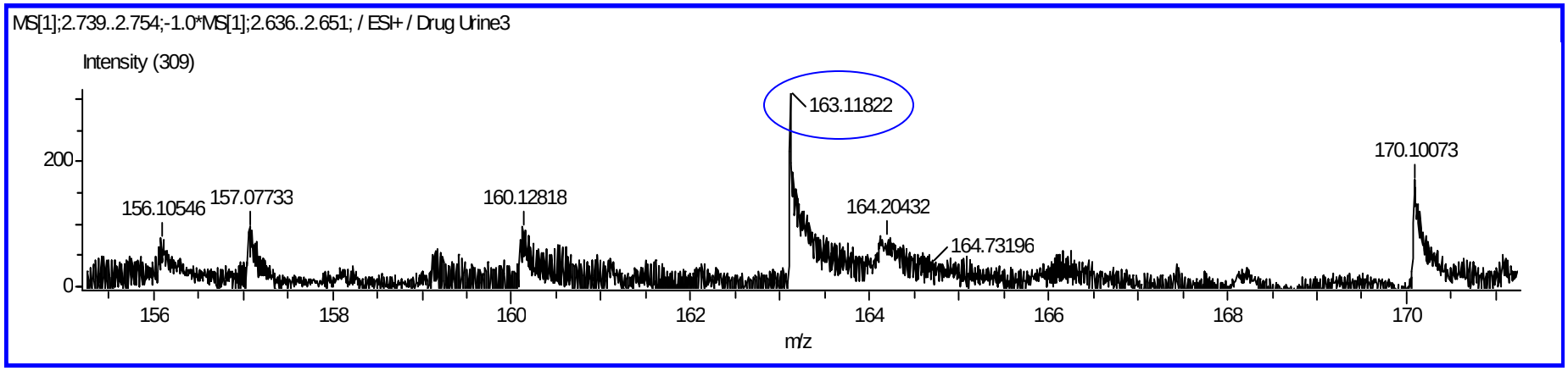


B-11.11. Nicotine 3.125 μg/mL 163.122



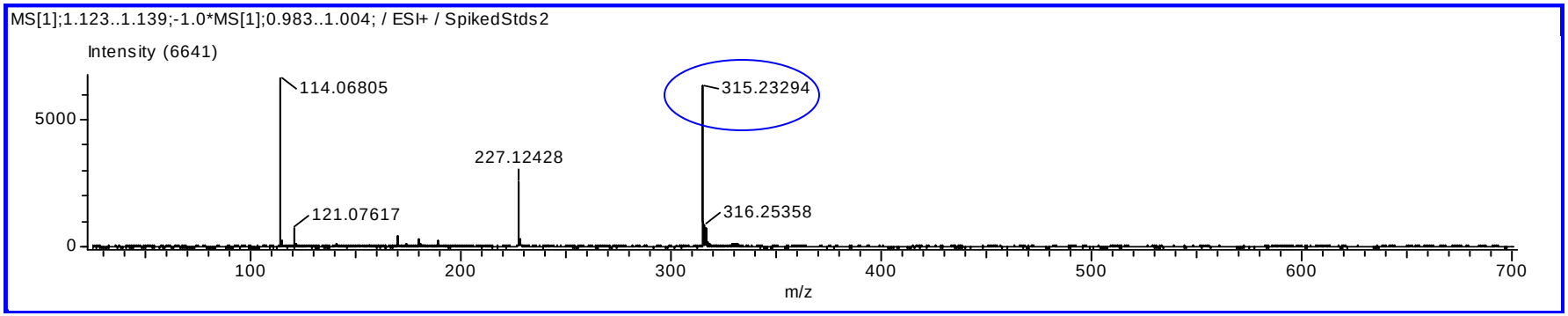
B-11. Miscellaneous (continued)

B-11.12. Nicotine 1.56 μg/mL 163.122

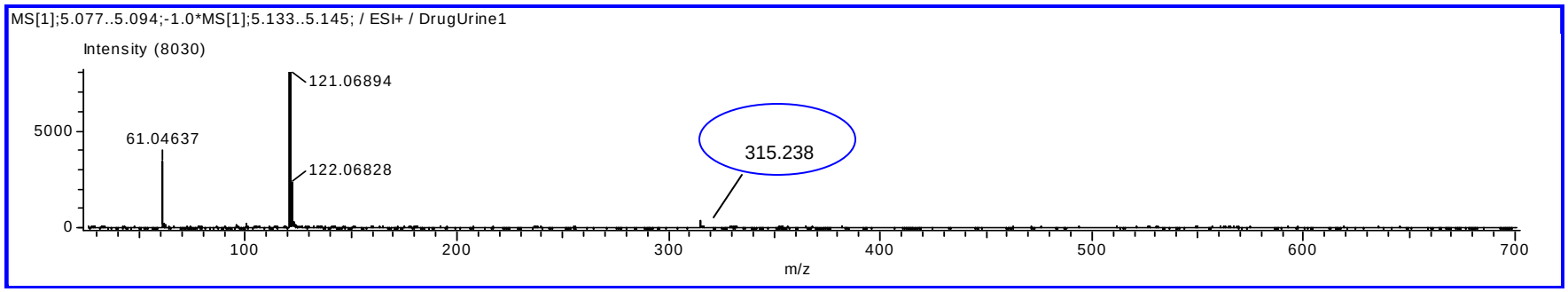


B-11. Miscellaneous (continued)

B-11.13. Δ9-THC 100 μg/mL 315.231

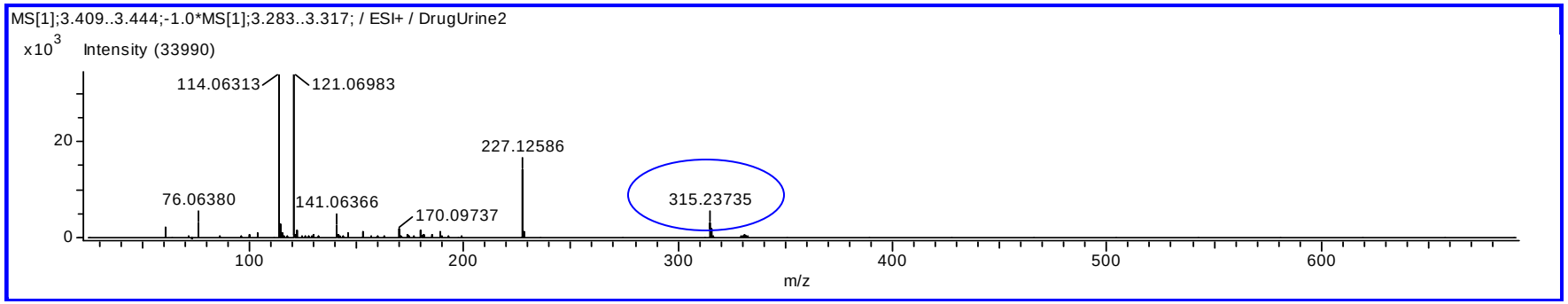


B-11.14. Δ9-THC 12.5 μg/mL 315.231

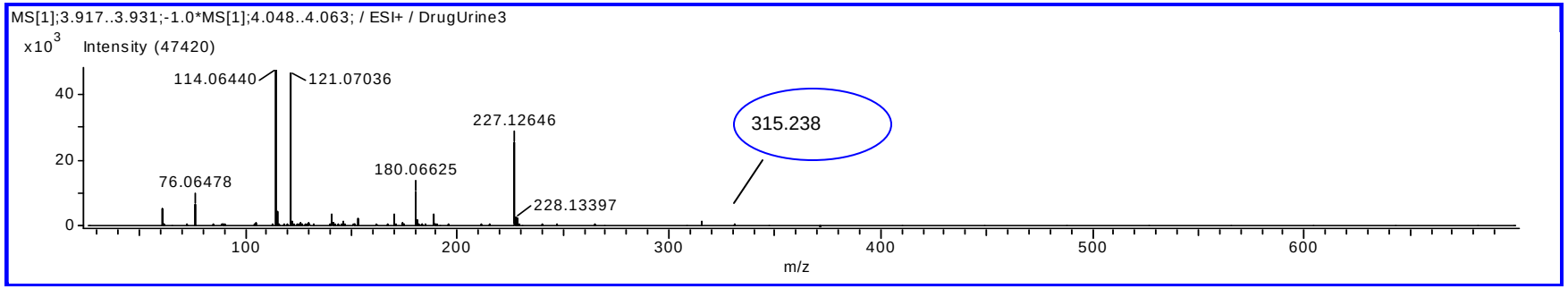


B-11. Miscellaneous (continued)

B-11.15. Δ9-THC 6.25 μg/mL 315.231

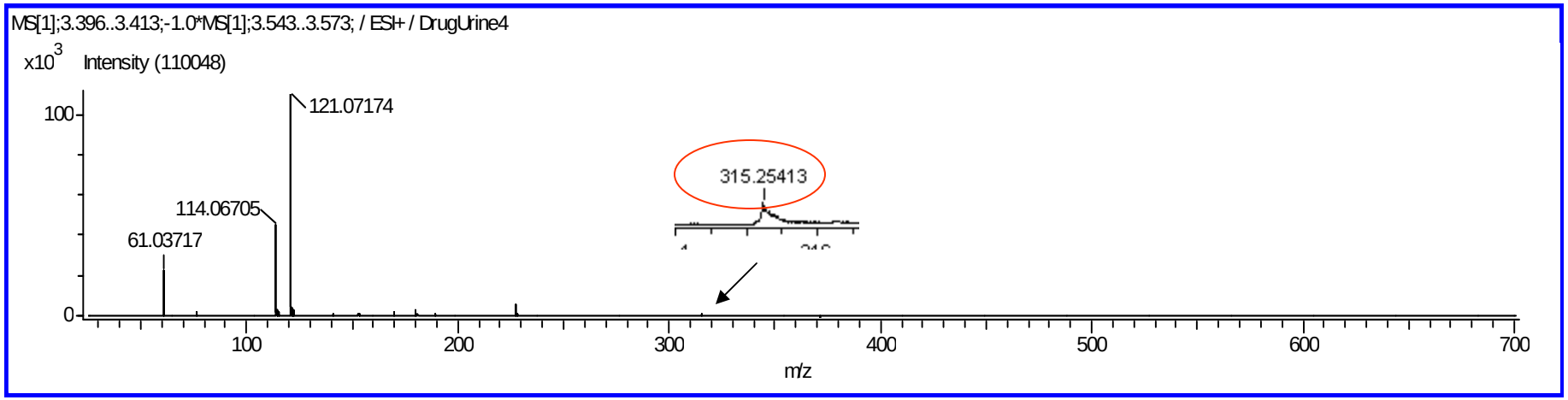


B-11.16. Δ9-THC 3.125 μg/mL 315.231



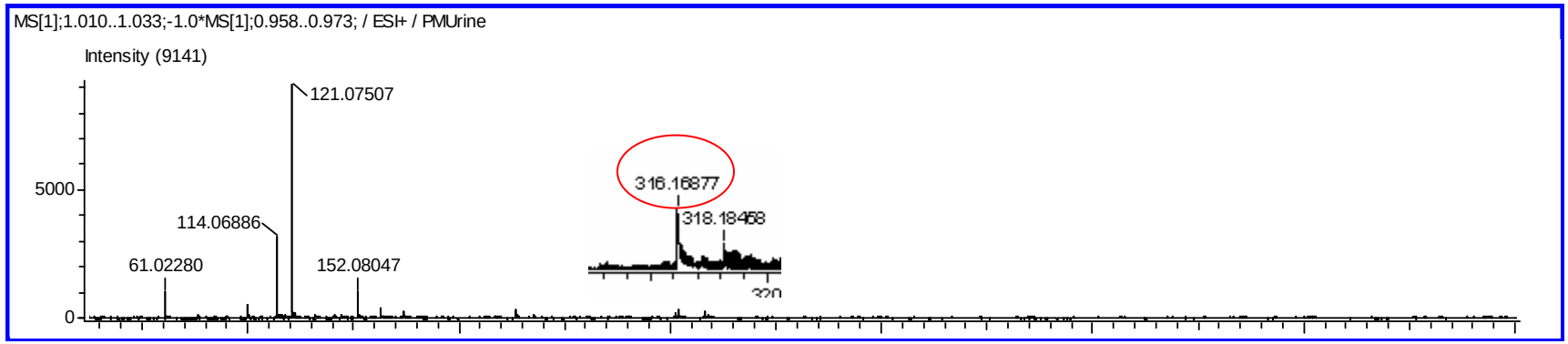
B-11. Miscellaneous (continued)

B-11.17. Δ9-THC 1.56 μg/mL 315.231

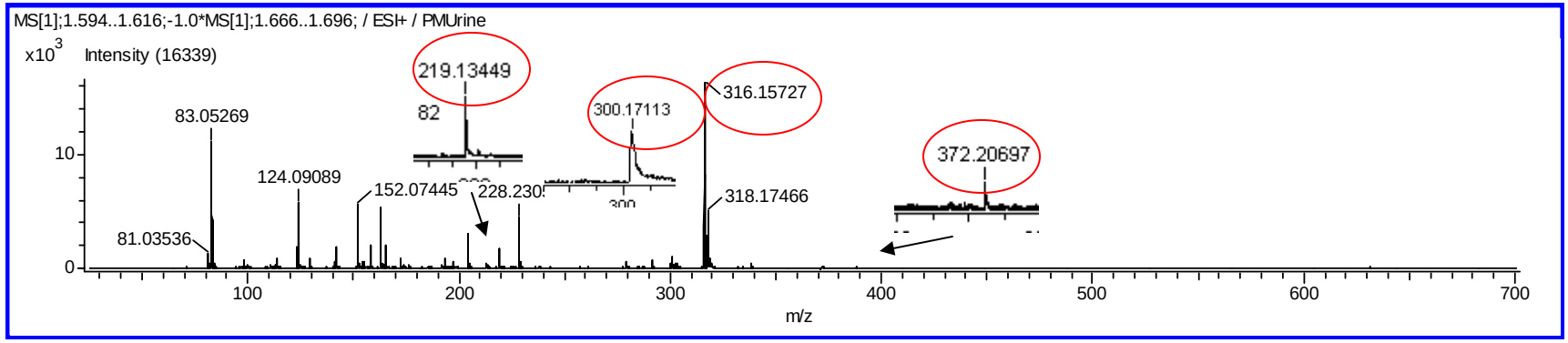


B-12. Postmortem Urine

B-12.1. Unextracted



B-12.2. Extracted in 3:1 n-butylchloride:ether/concentrated in 100μL ACN



Medical Examiner Toxicology Lab Report
Oxycodone-316.154
Hydrocodone-300.159
Meprobamate-219.133
Promethazine-340.226
Trazodone-372.158

Appendix C

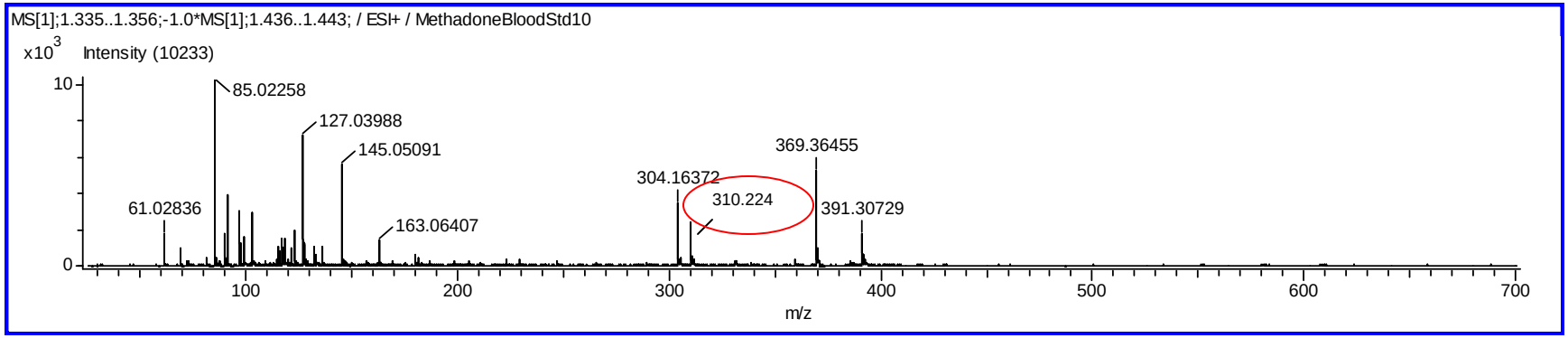
Drugs in Blood

Appendix C

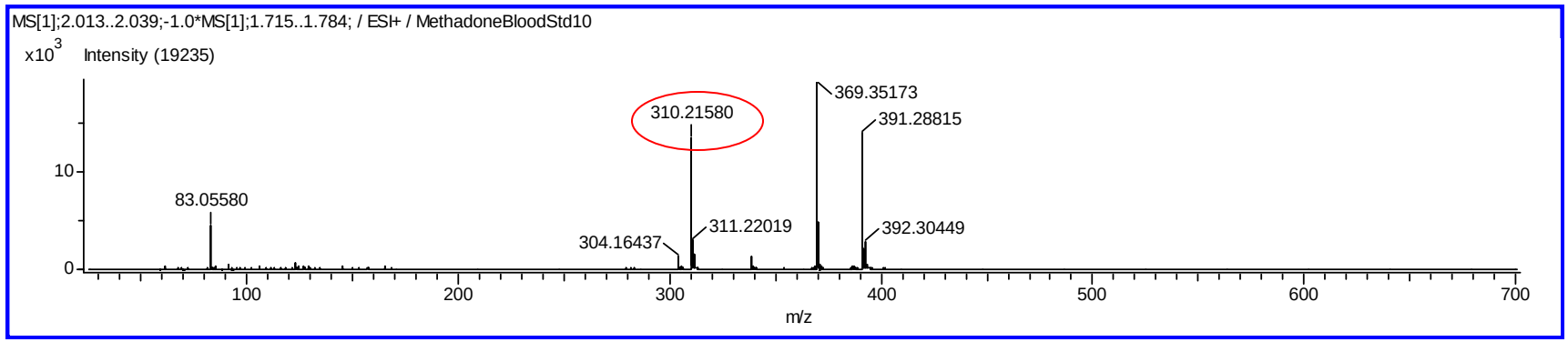
Drugs in Blood

C-1. Analgesics

C-1.1. 10 μg/mL Methadone in Blank Blood Extracted in 1:3 ACN

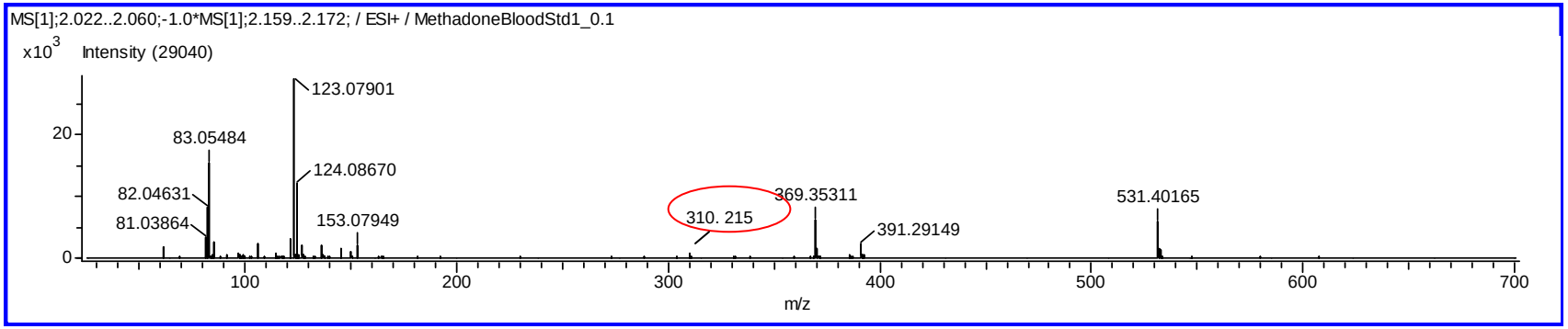


C-1.2. 10 μg/mL Methadone in Blank Blood Extracted in 1:3 ACN/dried/concentrated

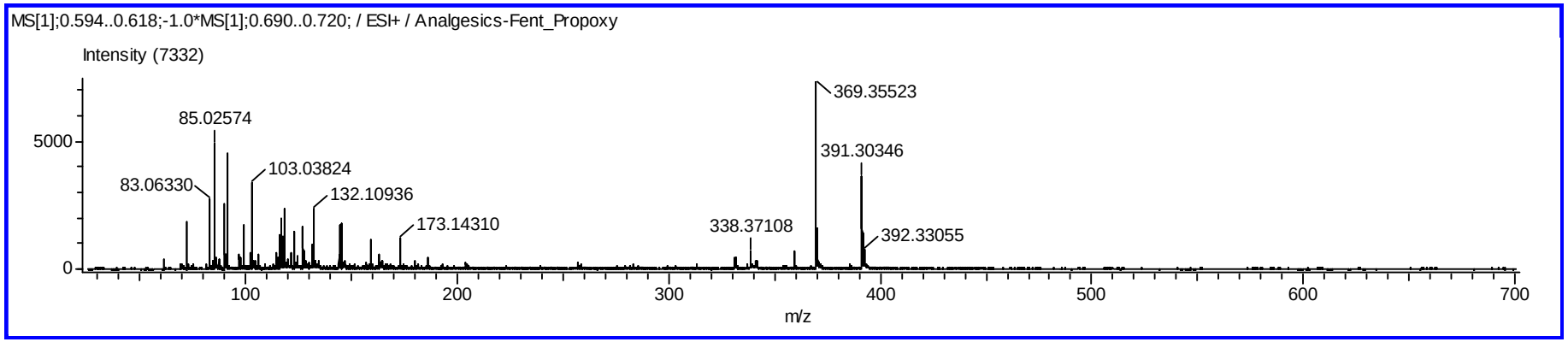


C-1. Analgesics (continued)

C-1.3. 1 µg/mL Methadone in Blank Blood Extracted in 1:3 ACN/dried/reconstituted in 100 µg/mL

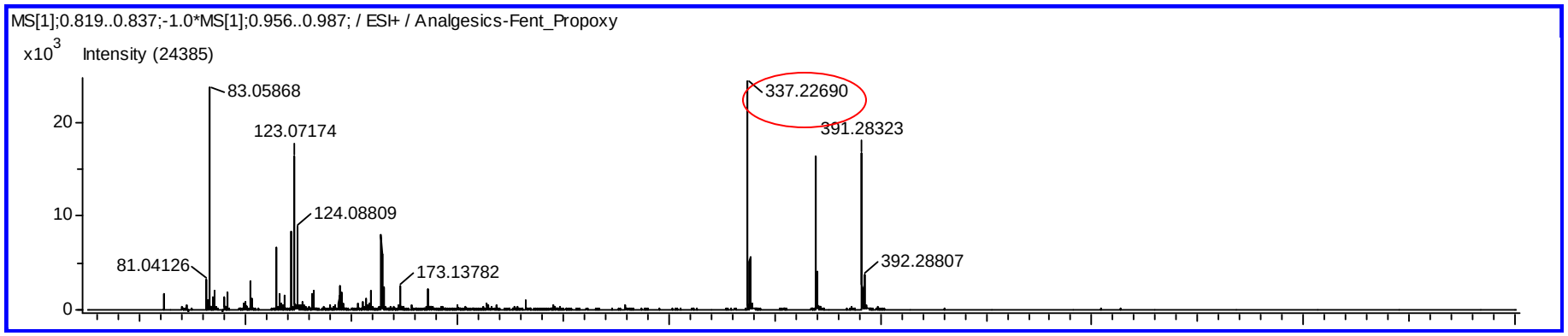


C-1.4. 10 µg/mL Fentanyl in Blank Blood Extracted 1:3 in ACN

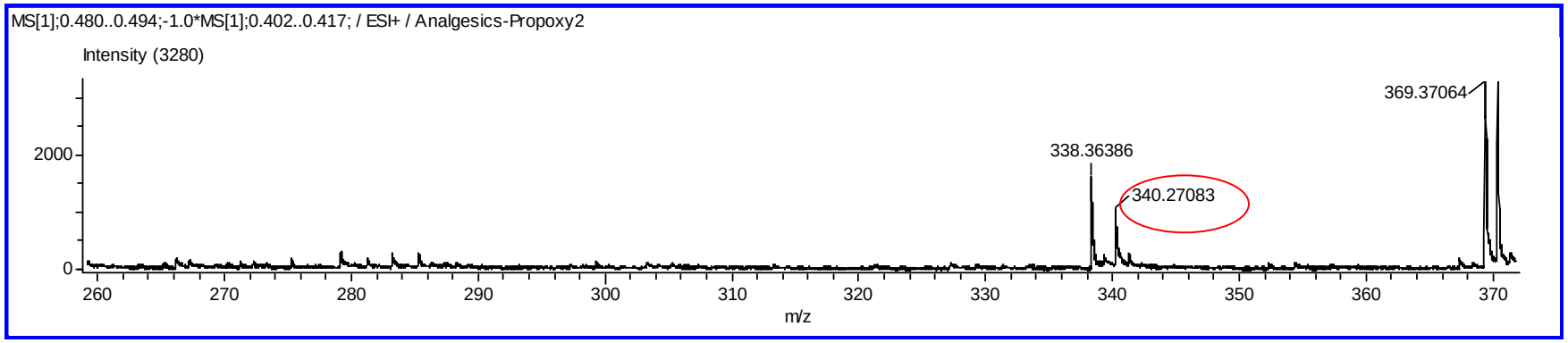


C-1. Analgesics (continued)

C-1.5. 10 μg/mL Fentanyl in Blank Blood Extracted in 1:3 ACN/dried/concentrated

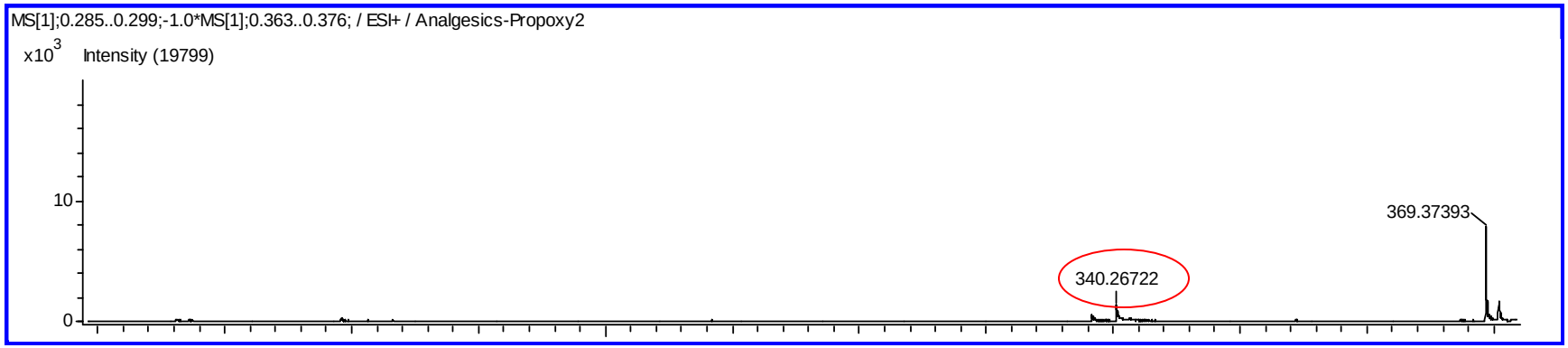


C-1.6. 10 μg/mL Propoxyphene in Blank Blood Extracted 1:3 in ACN



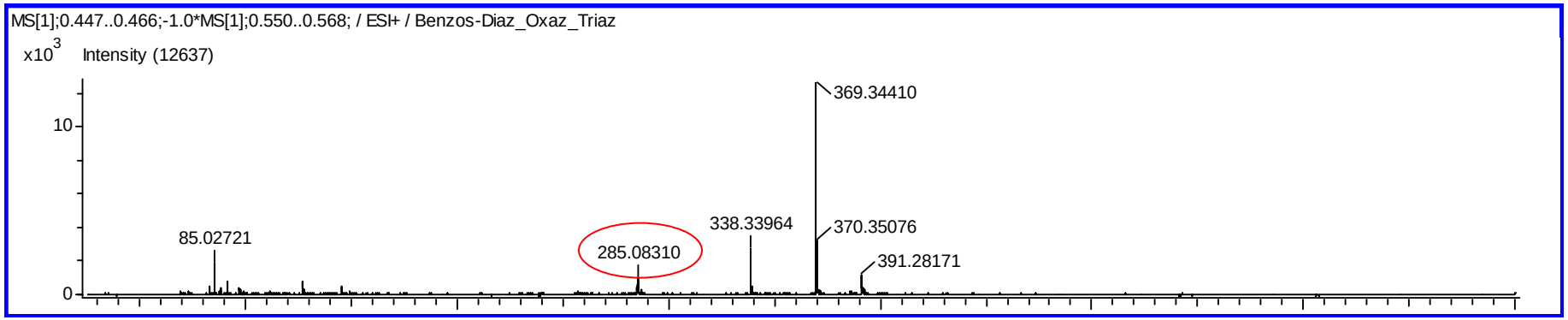
C-1. Analgesics (continued)

C-1.7. 10 μg/mL Propoxyphene in Blank Blood Extracted in 1:3 ACN/dried/concentrated

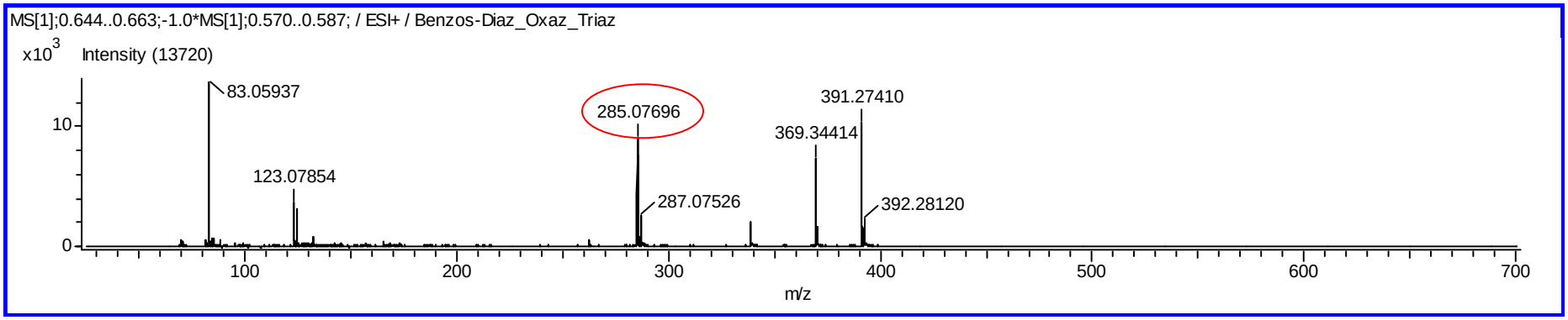


C-2. Benzodiazepines

C-2.1. 100 μg/mL Diazepam in Blank Blood Extracted 1:3 in ACN

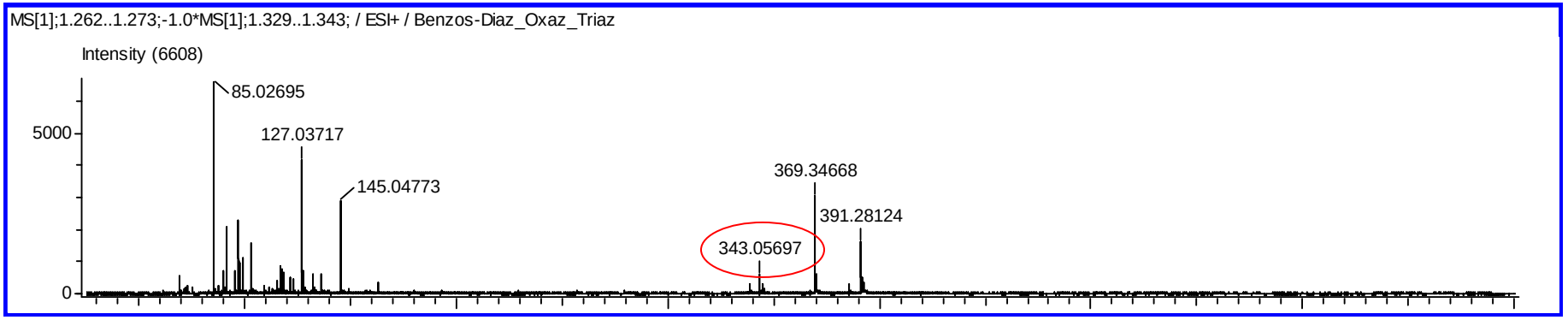


C-2.2. 100 μg/mL Diazepam in Blank Blood Extracted in 1:3 ACN/dried/concentrated

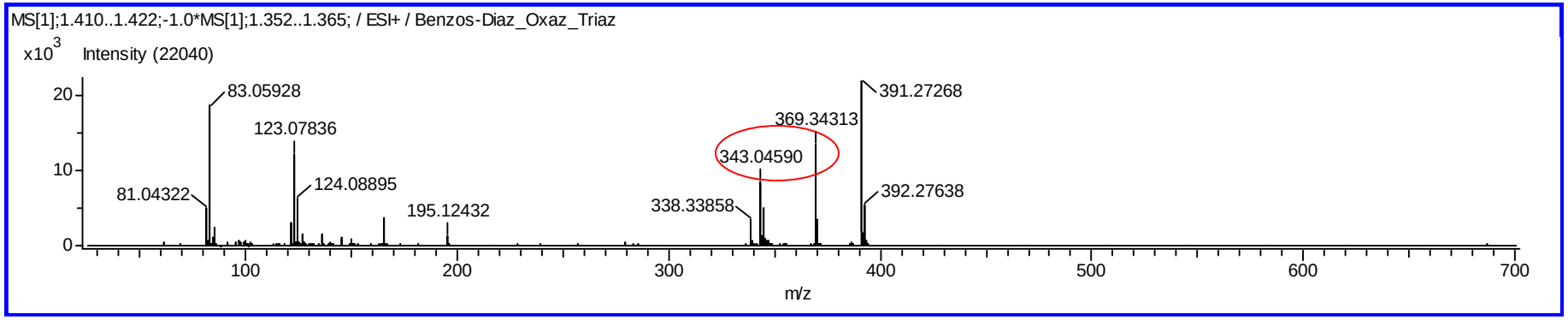


C-2. Benzodiazepines (continued)

C-2.3. 100 μg/mL Triazolam in Blank Blood Extracted 1:3 in ACN

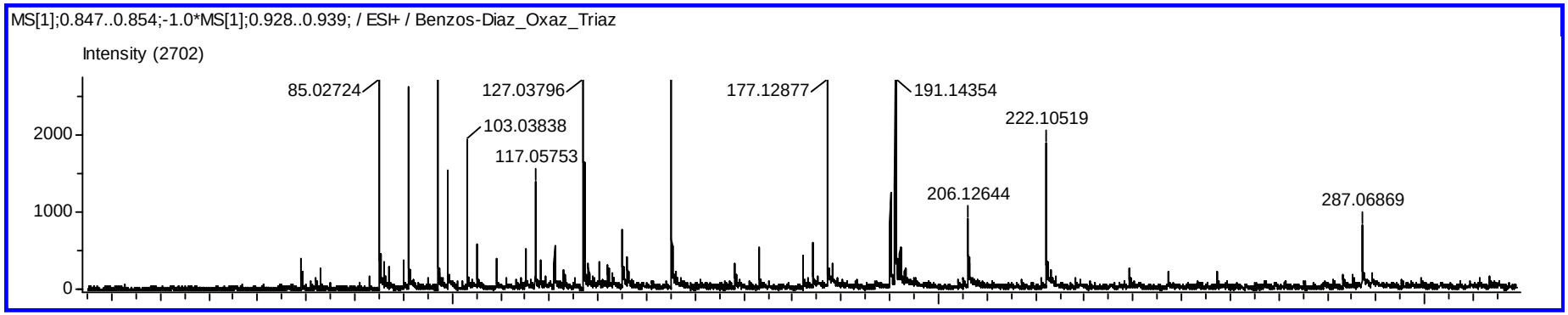


C-2.4. 100 μg/mL Triazolam in Blank Blood Extracted in 1:3 ACN/dried/concentrated

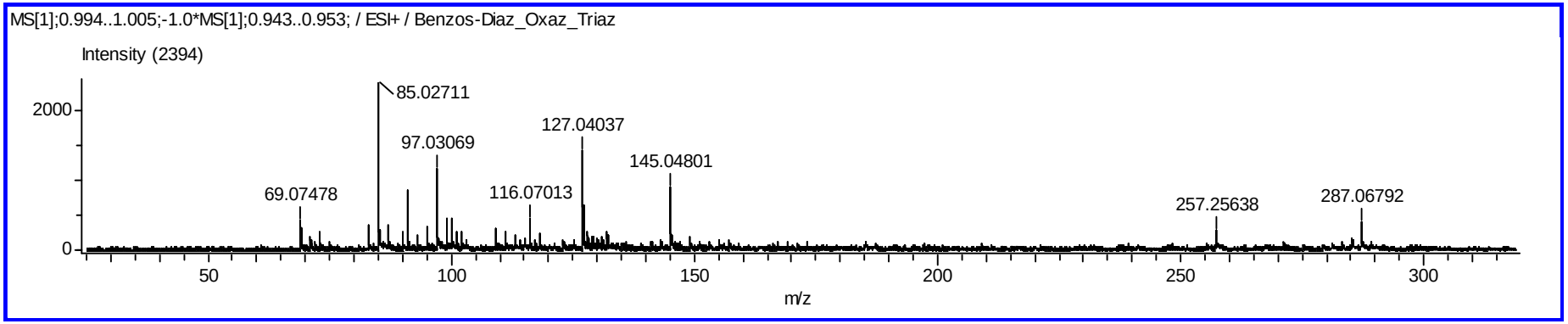


C-2. Benzodiazepines (continued)

C-2.5. 100 μg/mL Oxazepam in Blank Blood Extracted 1:3 in ACN

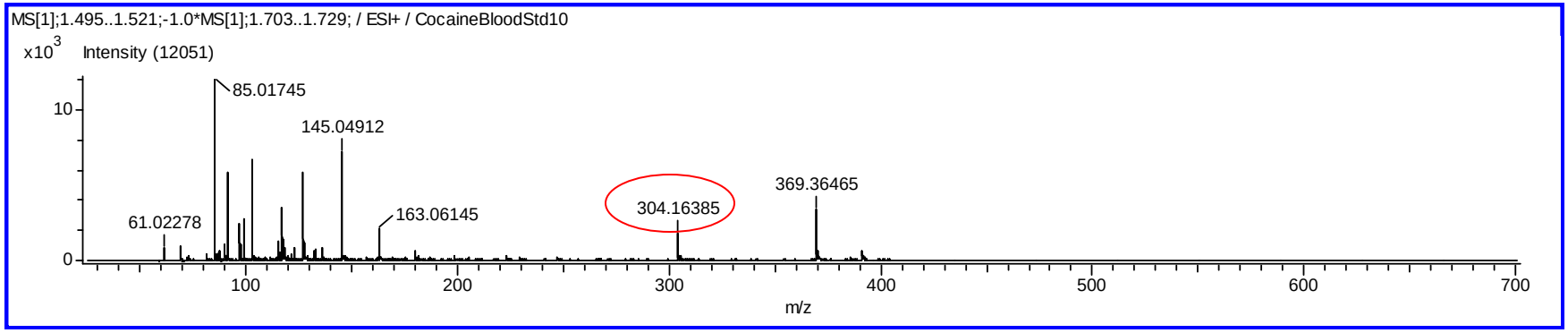


C-2.6. 10 μg/mL Oxazepam in Blank Blood Extracted in 1:3 ACN/dried/concentrated

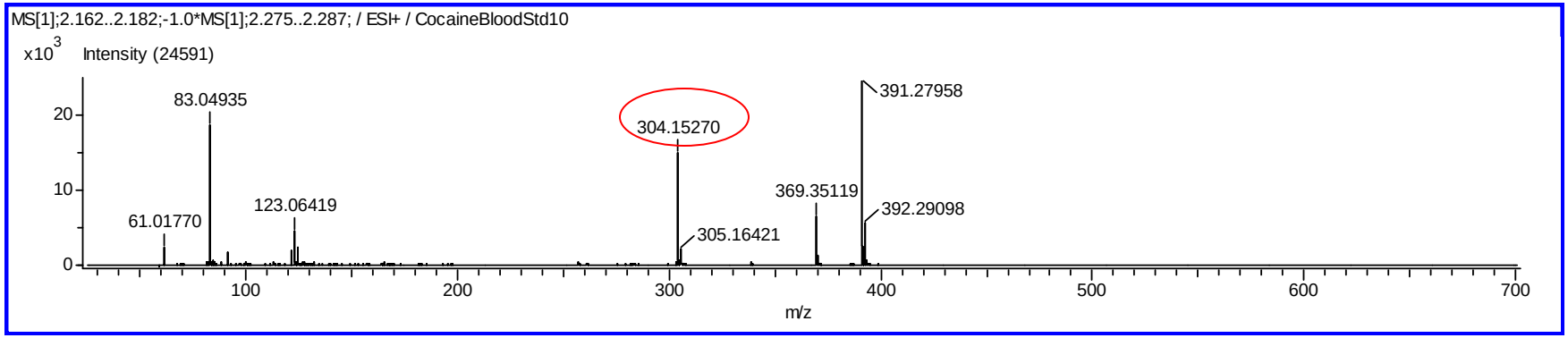


C-3. Cocaine and Metabolites

C-3.1. 10 µg/mL Cocaine in Blank Blood Extracted 1:3 in ACN

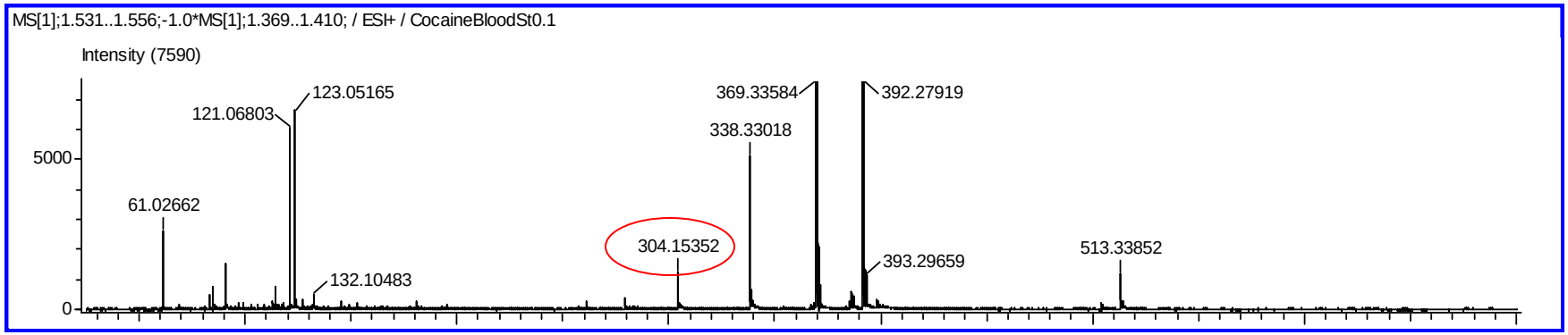


C-3.2. 10 µg/mL Cocaine in Blank Blood Extracted in 1:3 ACN/dried/reconstituted in 100 µg/mL



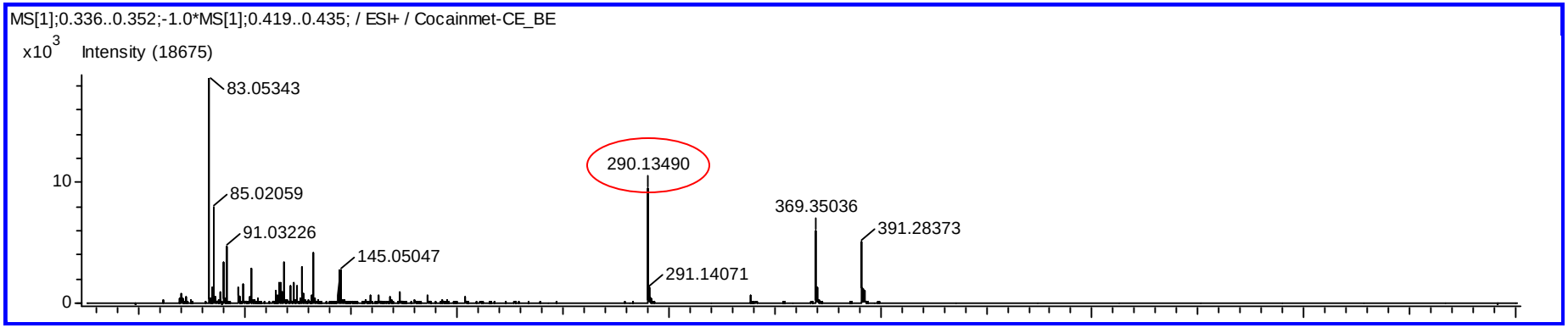
C-3. Cocaine and Metabolites (continued)

C-3.3. 0.1 µg/mL Cocaine in Blank Blood Extracted in 1:3 ACN/dried/reconstituted in 100 µg/mL

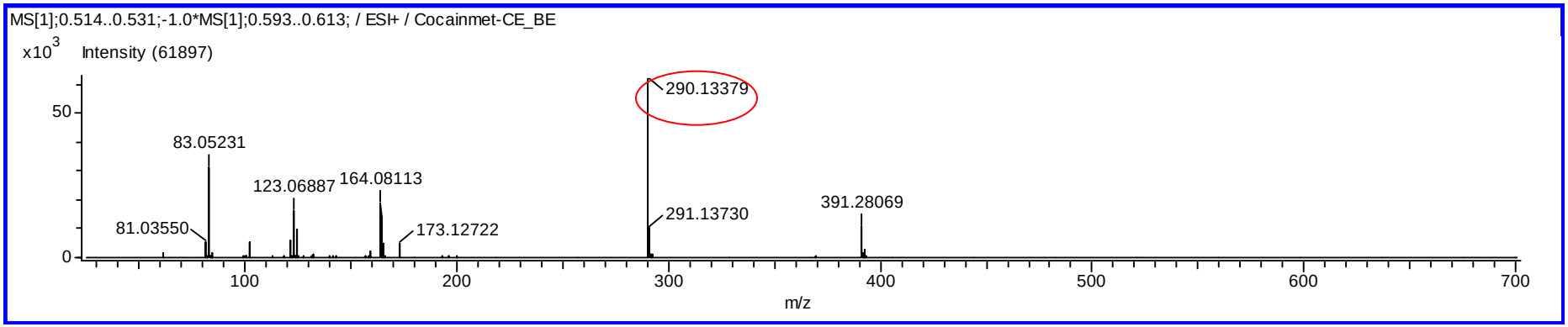


C-3. Cocaine and Metabolites (continued)

C-3.4. 10 μg/mL Benzoylecgonine in Blank Blood Extracted 1:3 in ACN

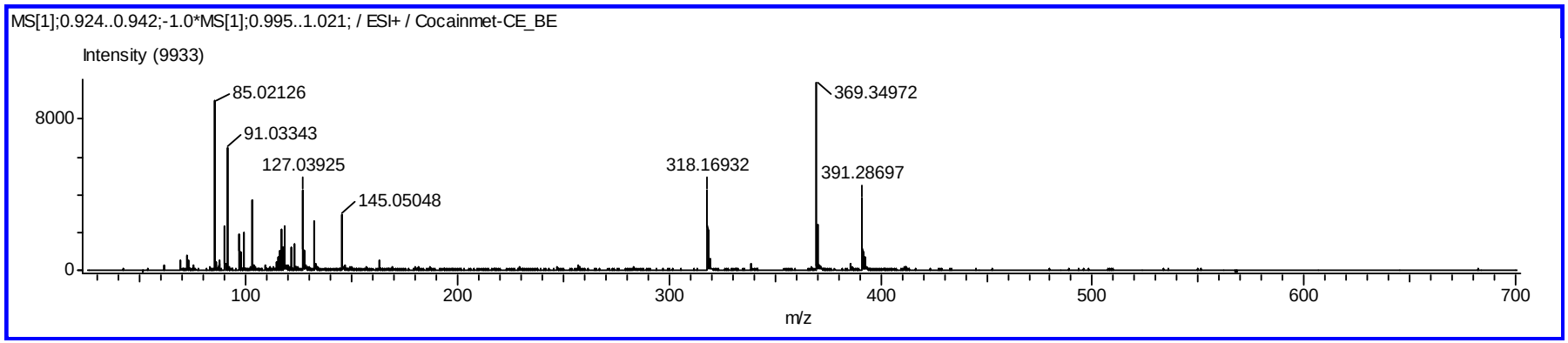


C-3.5. 10 μg/mL Benzoylecgonine in Blank Blood Extracted in 1:3 ACN/dried/concentration

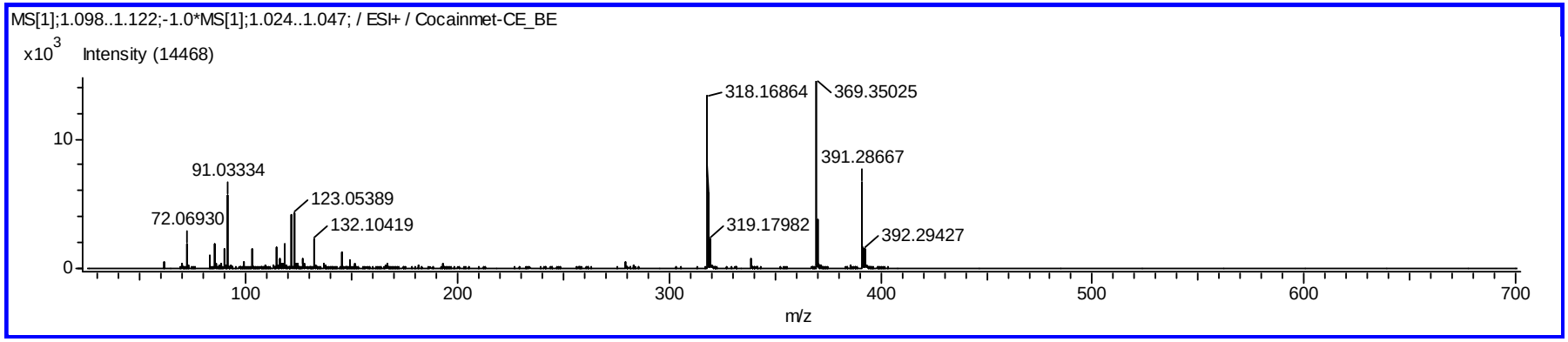


C-3. Cocaine and Metabolites (continued)

C-3.6. 10 μg/mL Cocaethylene in Blank Blood Extracted 1:3 in ACN

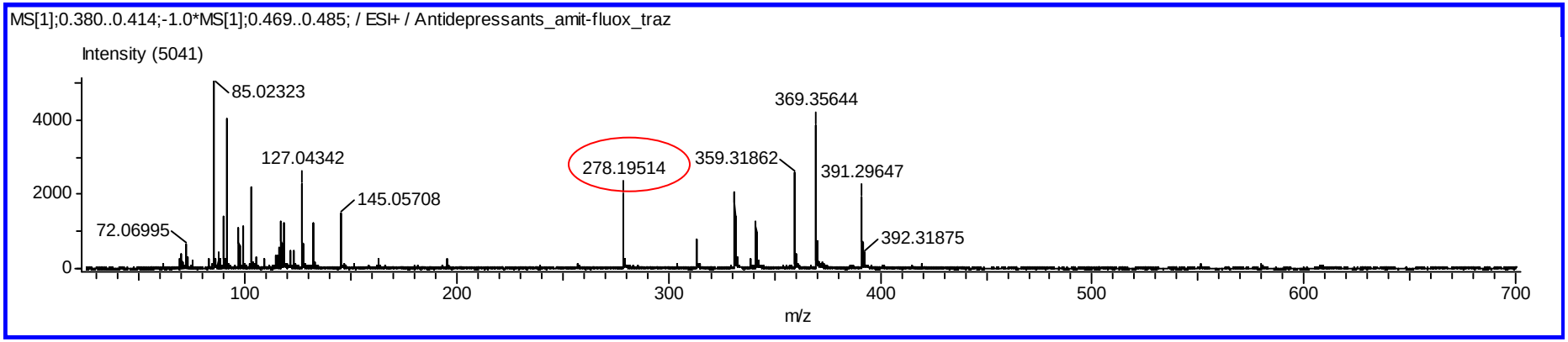


C-3.7. 10 μg/mL Cocaethylene in Blank Blood Extracted in 1:3 ACN/dried/concentration

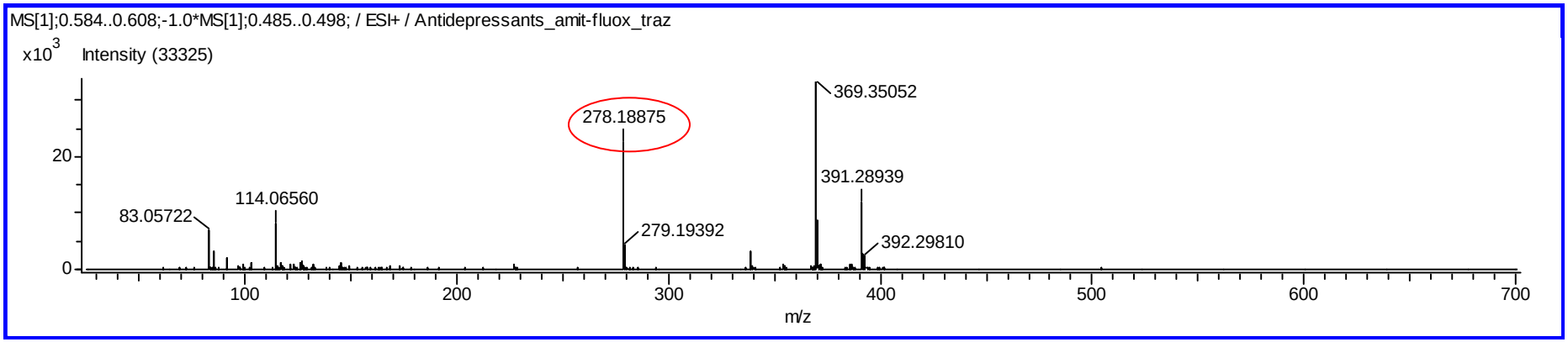


C-4. Antidepressants

C-4.1. 10 μg/mL Amitriptyline in Blank Blood Extracted 1:3 in ACN

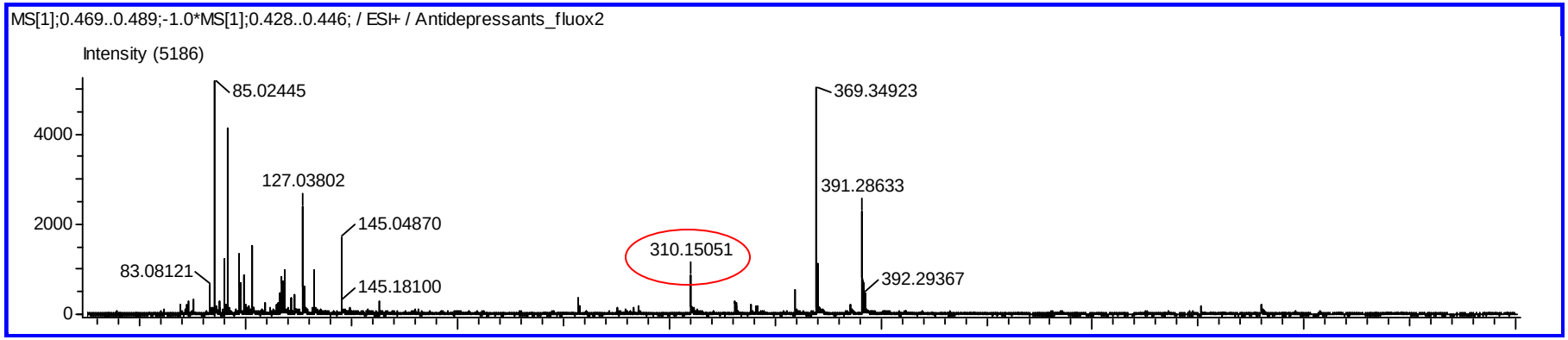


C-4.2. 10 μg/mL Amitriptyline in Blank Blood Extracted in 1:3 ACN/dried/concentrated

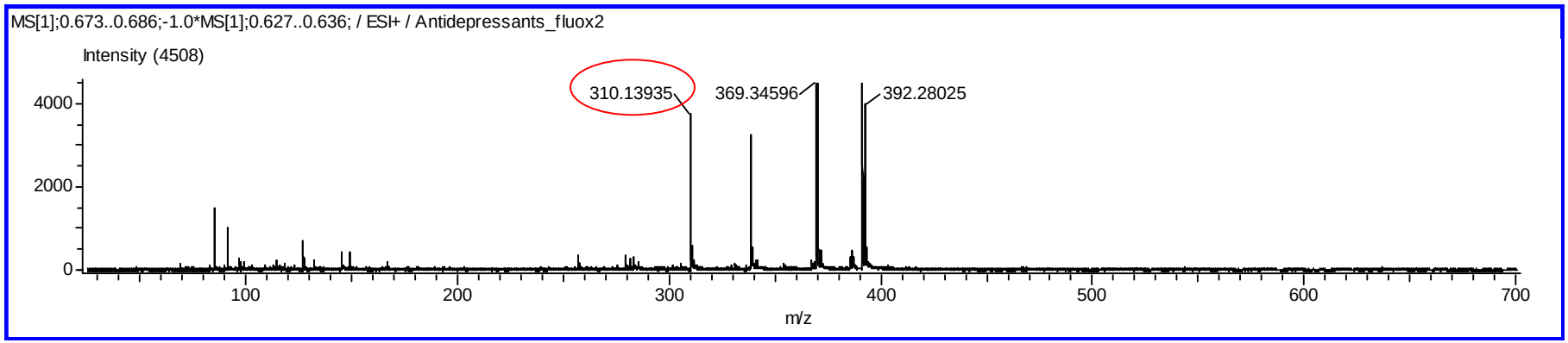


C-4. Antidepressants (continued)

C-4.3. 10 μg/mL Fluoxetine in Blank Blood Extracted 1:3 in ACN

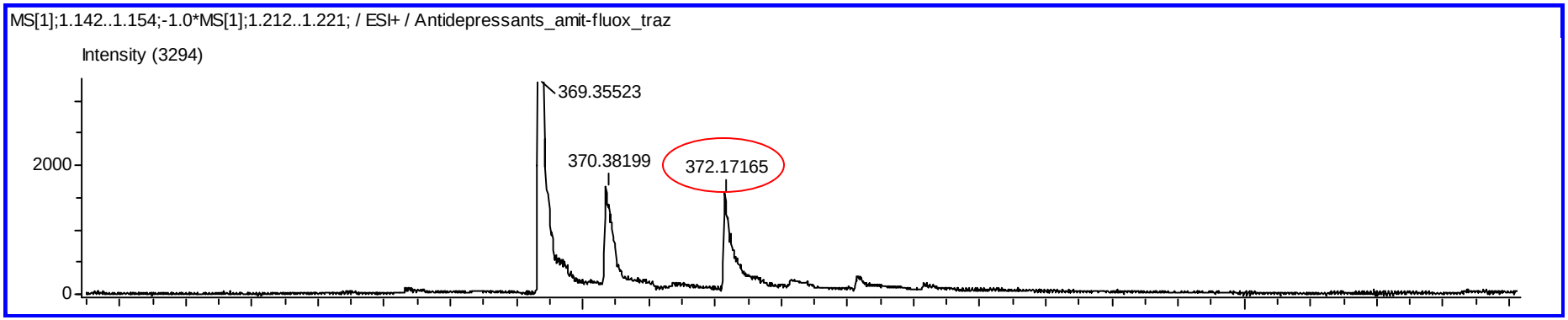


C-4.4. 10 μg/mL Fluoxetine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

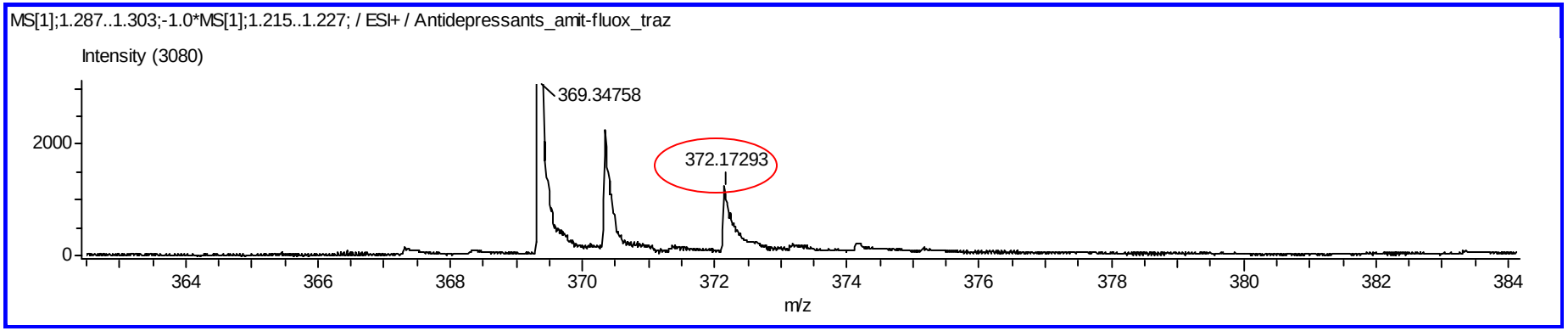


C-4. Antidepressants (continued)

C-4.5. 10 μg/mL Trazodone in Blank Blood Extracted 1:3 in ACN

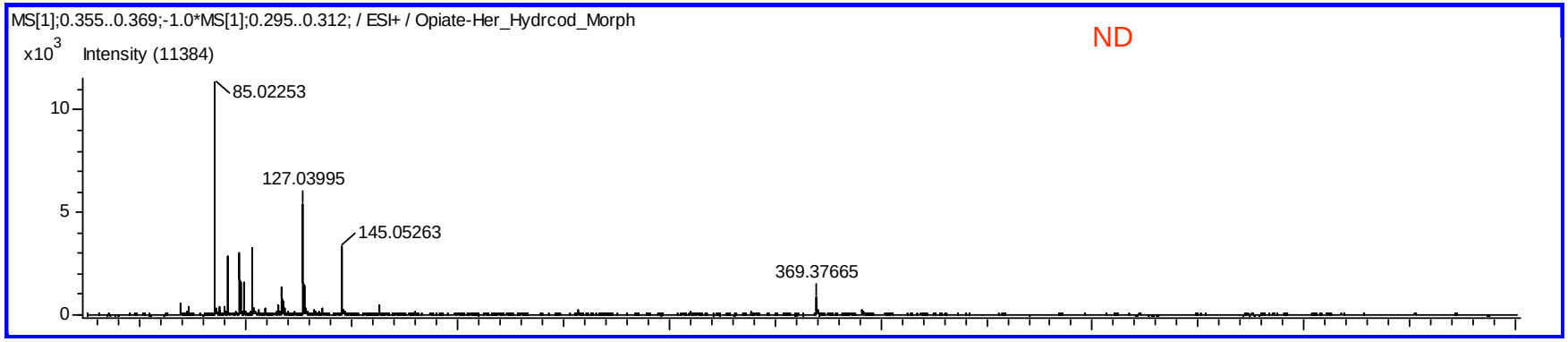


C-4.6. 10 μg/mL Trazodone in Blank Blood Extracted in 1:3 ACN/dried/concentrated

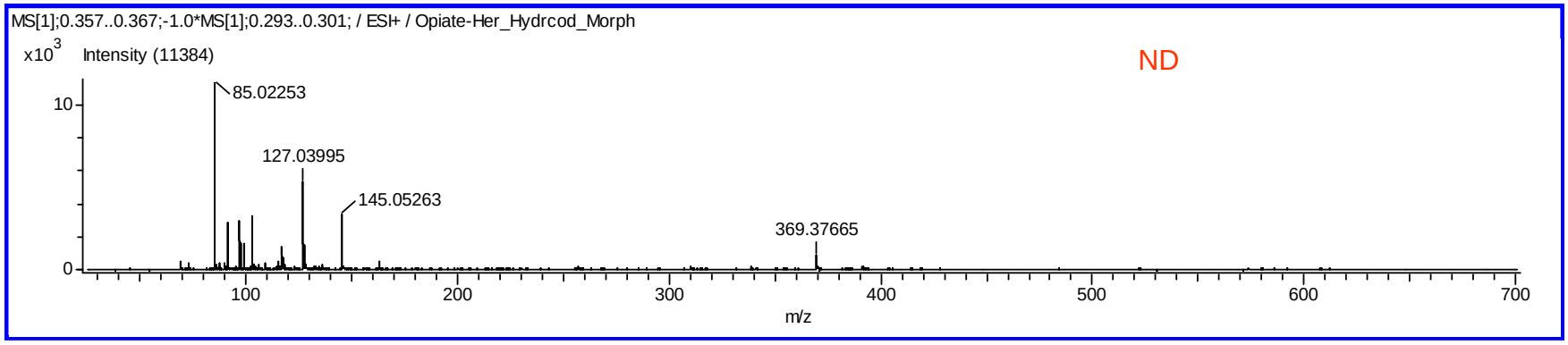


C-5. Opiates

C-5.1. 10 μg/mL Heroin in Blank Blood Extracted 1:3 in ACN

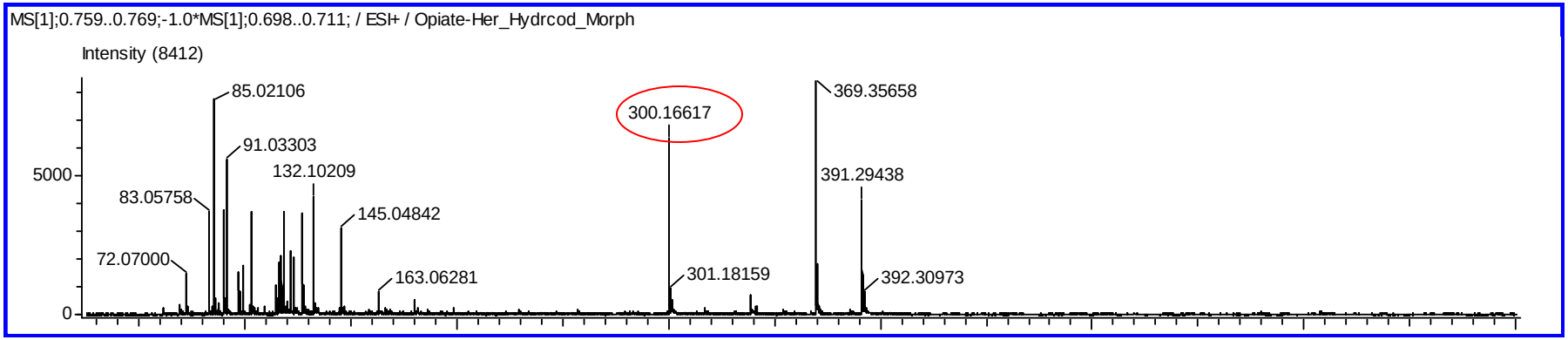


C-5.2. 10 μg/mL Heroin in Blank Blood Extracted in 1:3 ACN/dried/concentrated

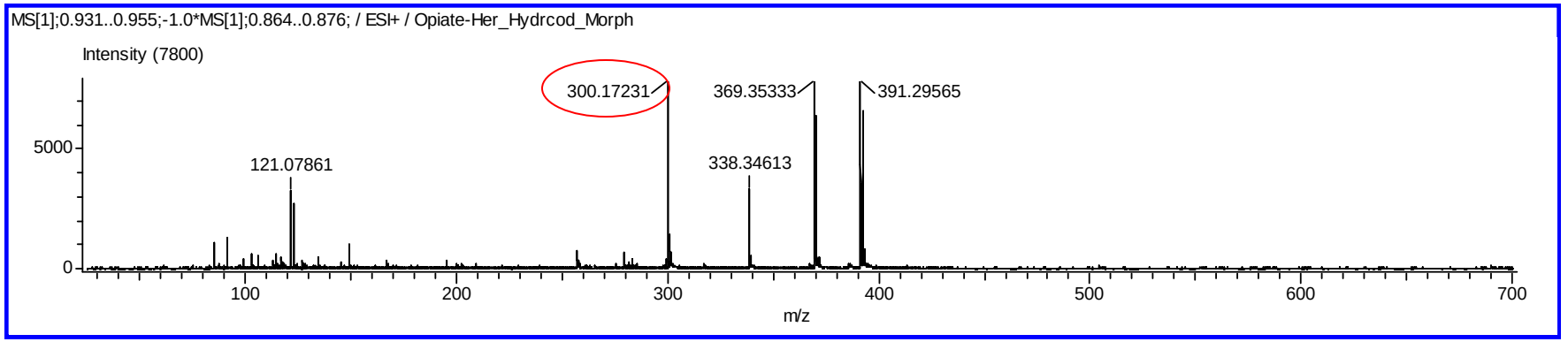


C-5. Opiates (continued)

C-5.3. 10 μg/mL Hydrocodone in Blank Blood Extracted 1:3 in ACN

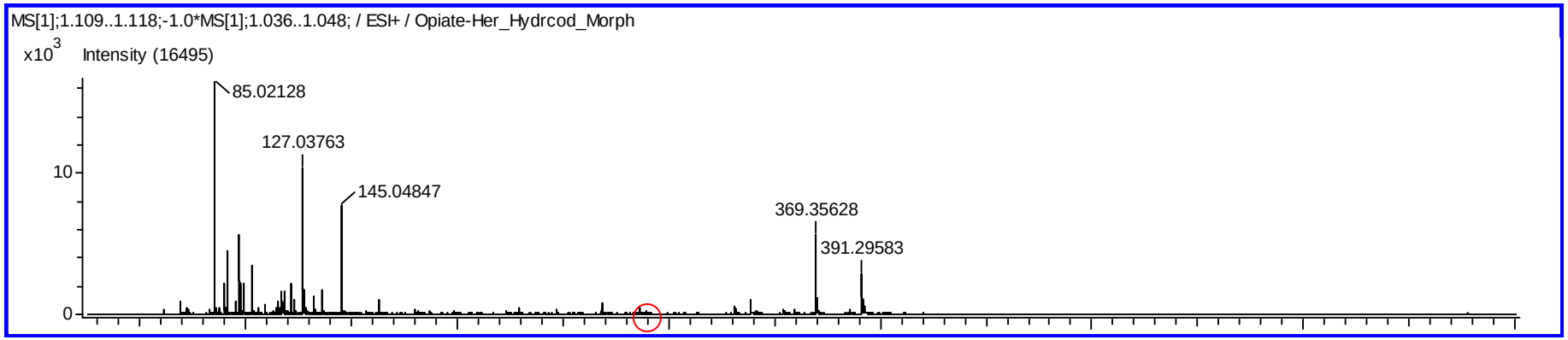


C-5.4. 10 μg/mL Hydrocodone in Blank Blood Extracted in 1:3 ACN/dried/concentrated

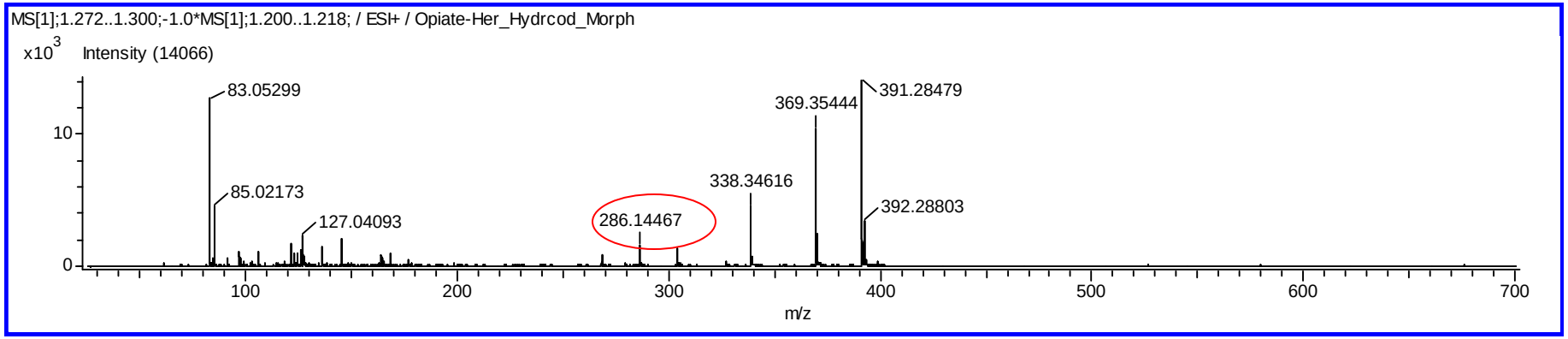


C-5. Opiates (continued)

C-5.5. 10 μg/mL Morphine in Blank Blood Extracted 1:3 in ACN

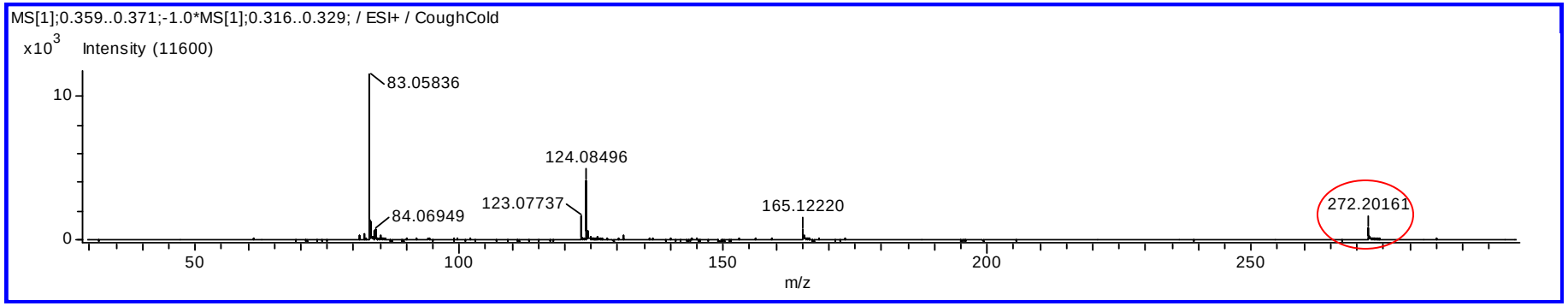


C-5.6. 10 μg/mL Morphine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

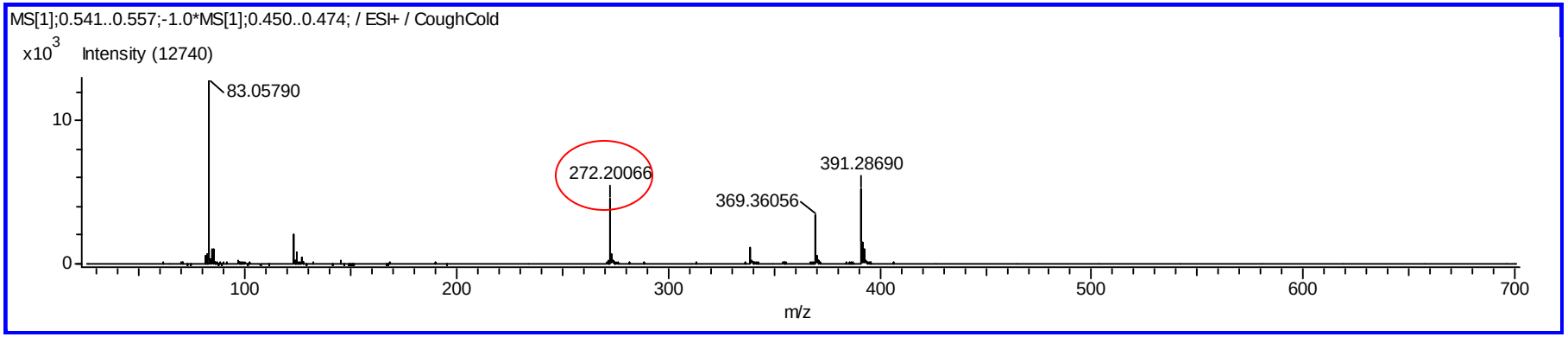


C-6. Allergy/Cold

C-6.1. 100 µg/mL Dextromethorphan in Blank Blood Extracted 1:3 in ACN

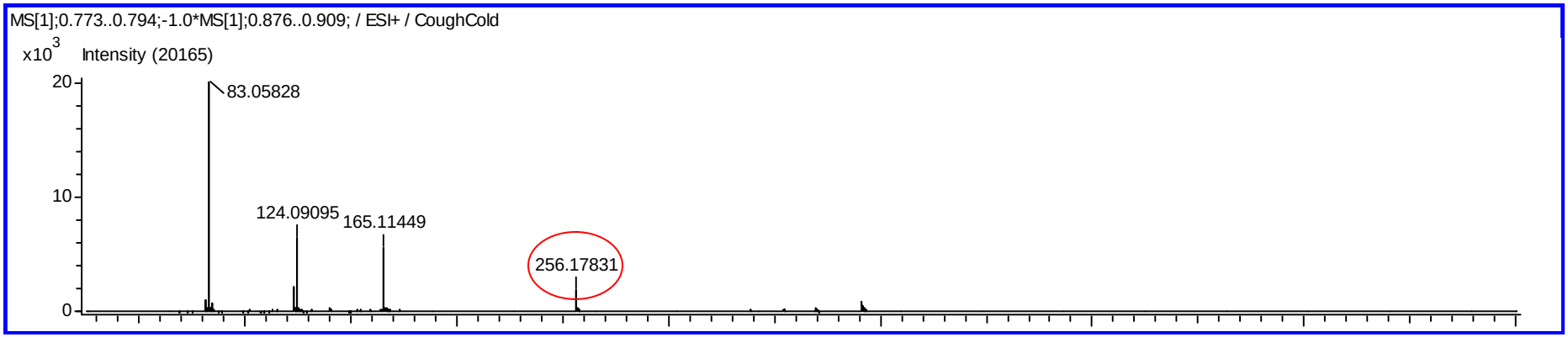


C-6.2. 100 mg/mL Dextromethorphan in Blank Blood Extracted in 1:3 ACN/dried/concentrated

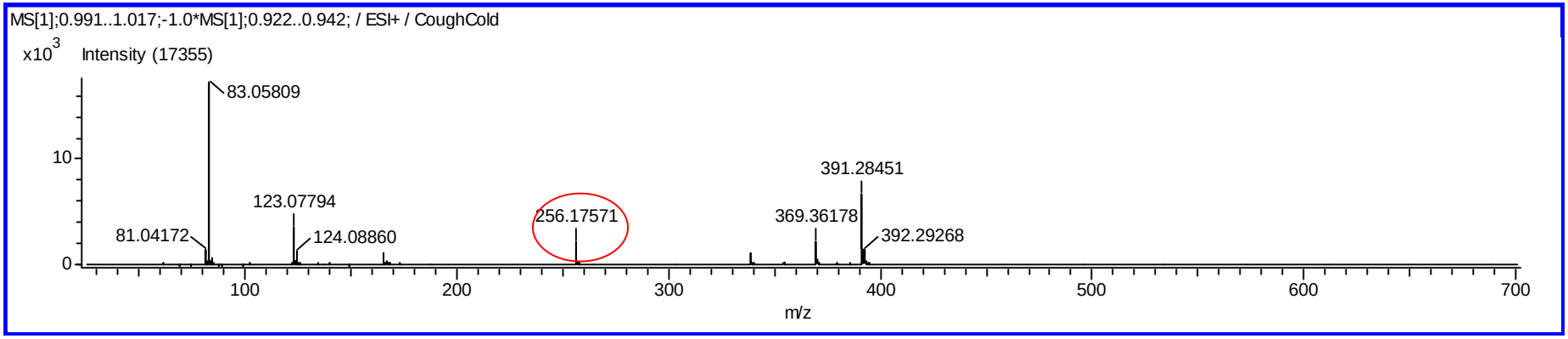


C-6. Allergy/Cold (continued)

C-6.3. 100 μg/mL Diphenhydramine in Blank Blood Extracted 1:3 in ACN

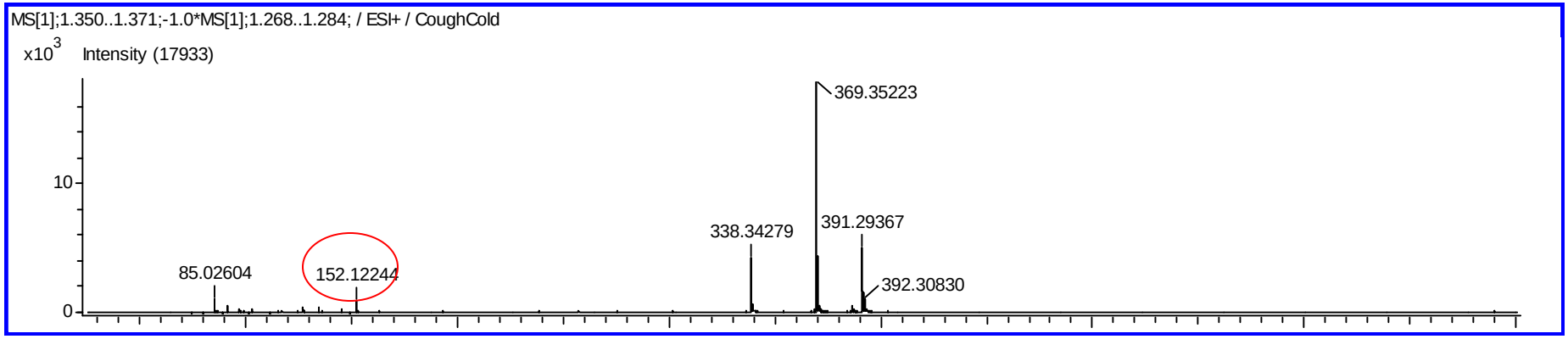


C-6.4. 100 μg/mL Diphenhydramine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

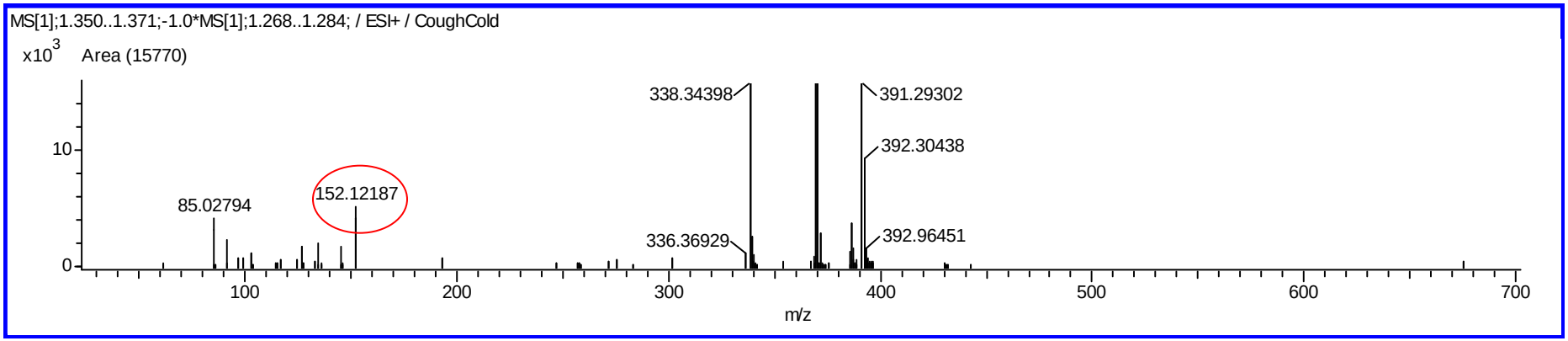


C-6. Allergy/Cold (continued)

C-6.5. 100 μg/mL Phenylpropanolamine in Blank Blood Extracted 1:3 in ACN

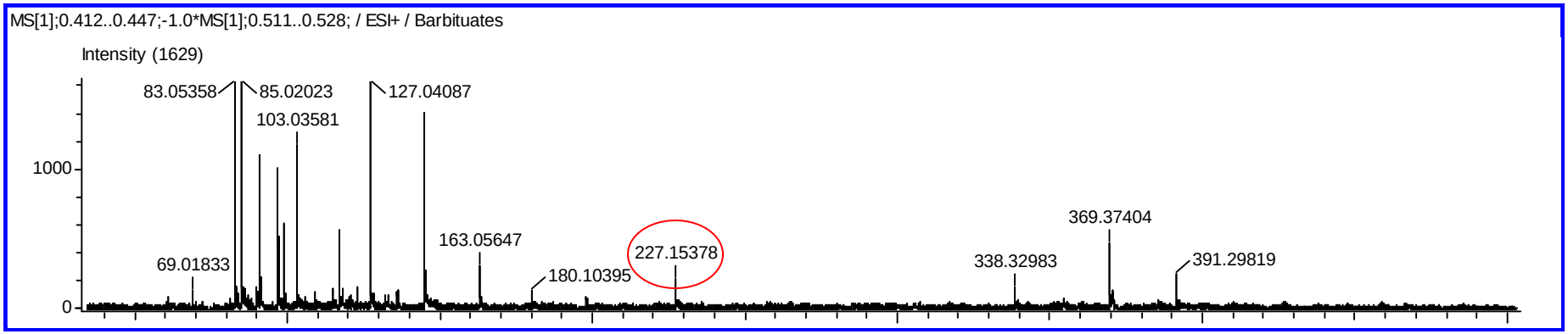


C-6.6. 100 μg/mL Phenylpropanolamine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

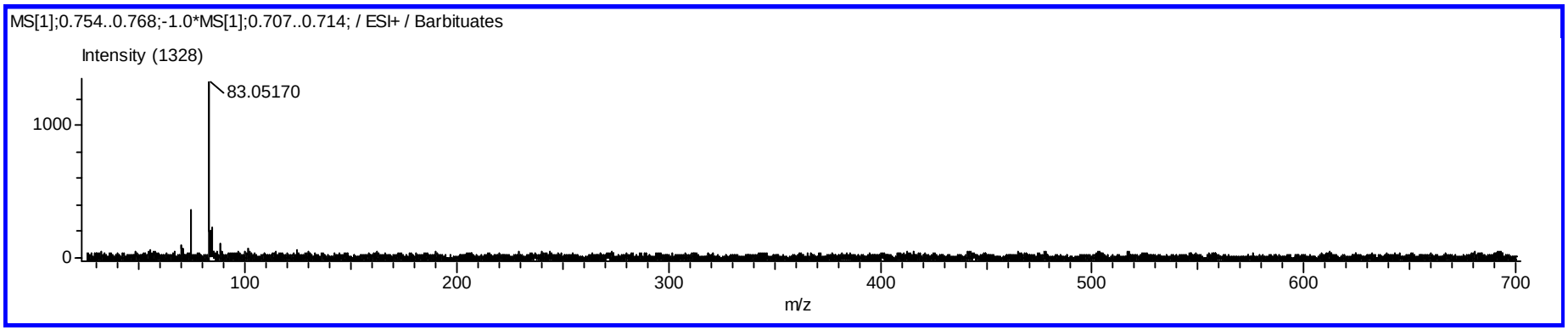


C-7. Barbiturates

C-7.1. 100 μg/mL Amobarbital in Blank Blood Extracted 1:3 in ACN



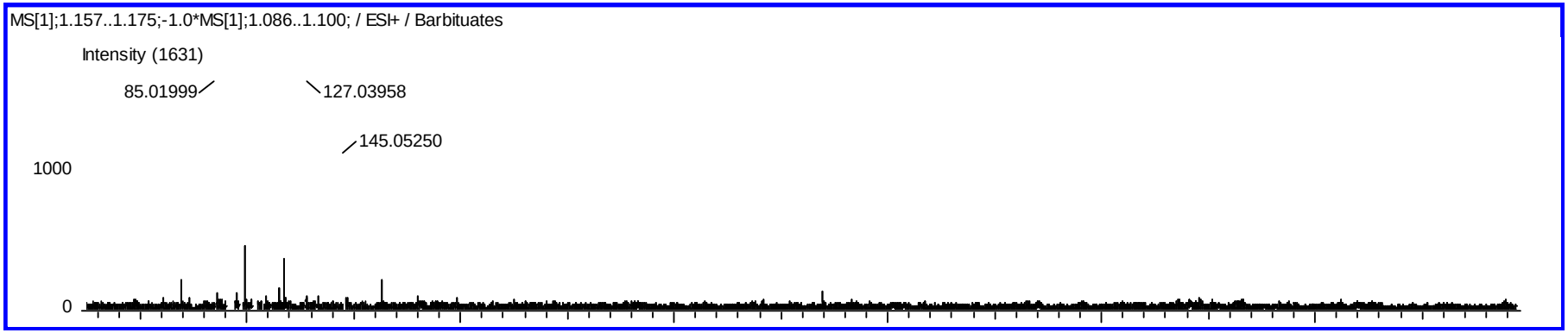
C-7.2. 100 μg/mL Amobarbital in Blank Blood Extracted in 1:3 ACN/dried/concentrated*



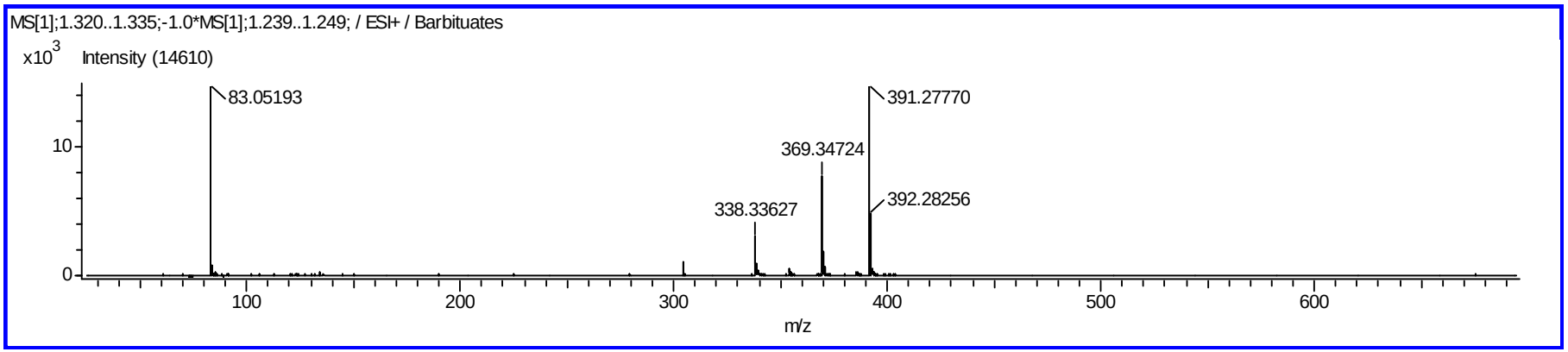
* Not detected

C-7. Barbiturates (continued)

C-7.3. 100 μg/mL Butalbital in Blank Blood Extracted 1:3 in ACN*



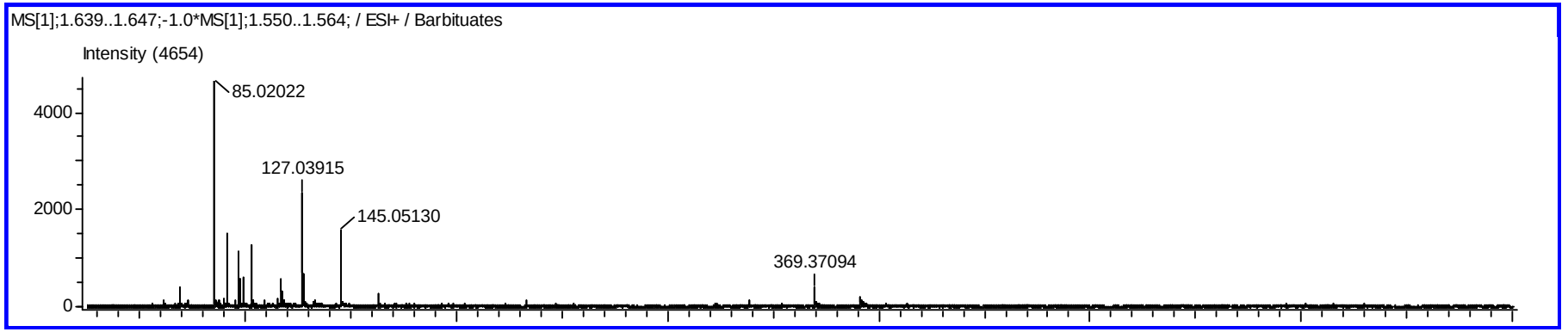
C-7.4. 100 μg/mL Butalbital in Blank Blood Extracted in 1:3 ACN/dried/concentrated*



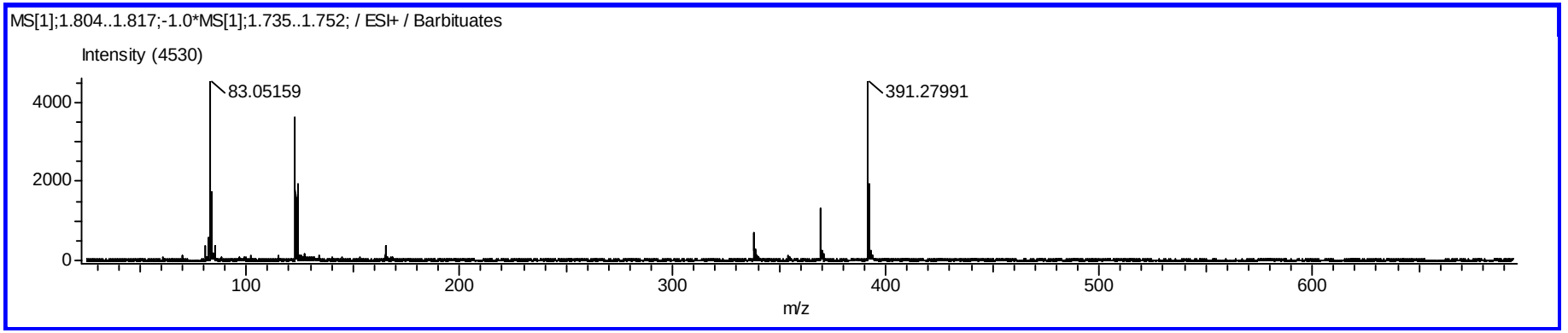
*Not detected

C-7. Barbiturates (continued)

C-7.5. 100 μg/mL Phenobarbital in Blank Blood Extracted 1:3 in ACN*



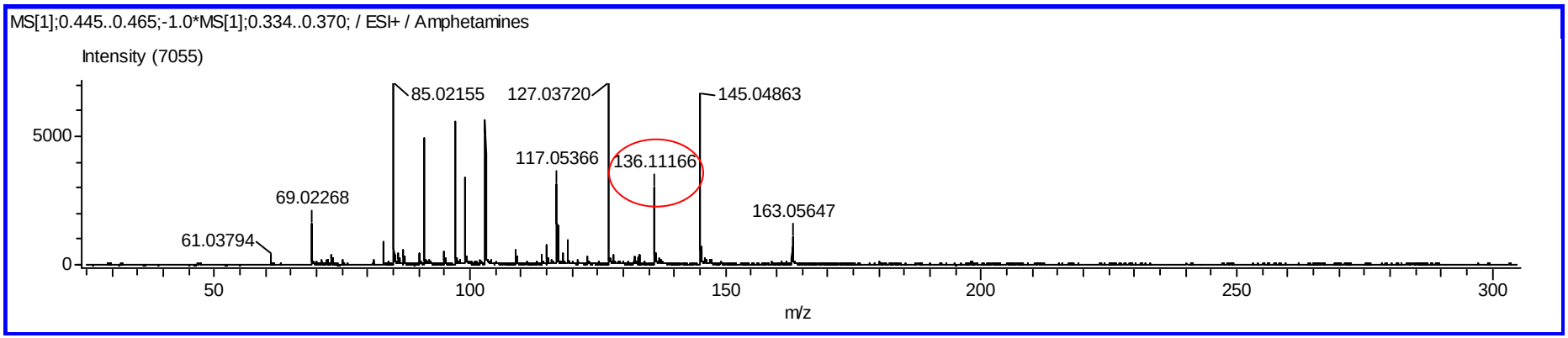
C-7.6. 100 μg/mL Phenobarbital in Blank Blood Extracted in 1:3 ACN/dried/concentrated*



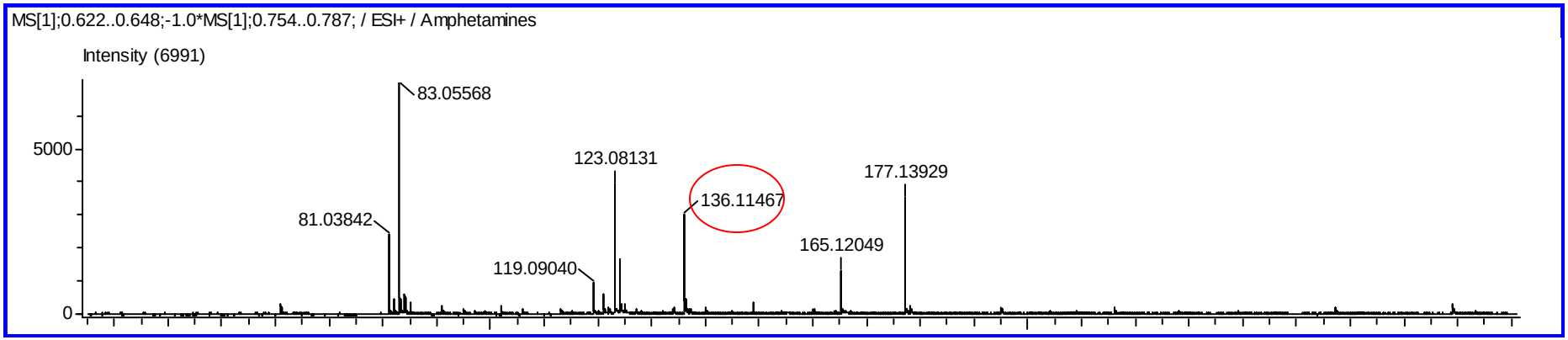
*Not detected

C-8. Amphetamines

C-8.1. 100 μg/mL Amphetamine in Blank Blood Extracted 1:3 in ACN

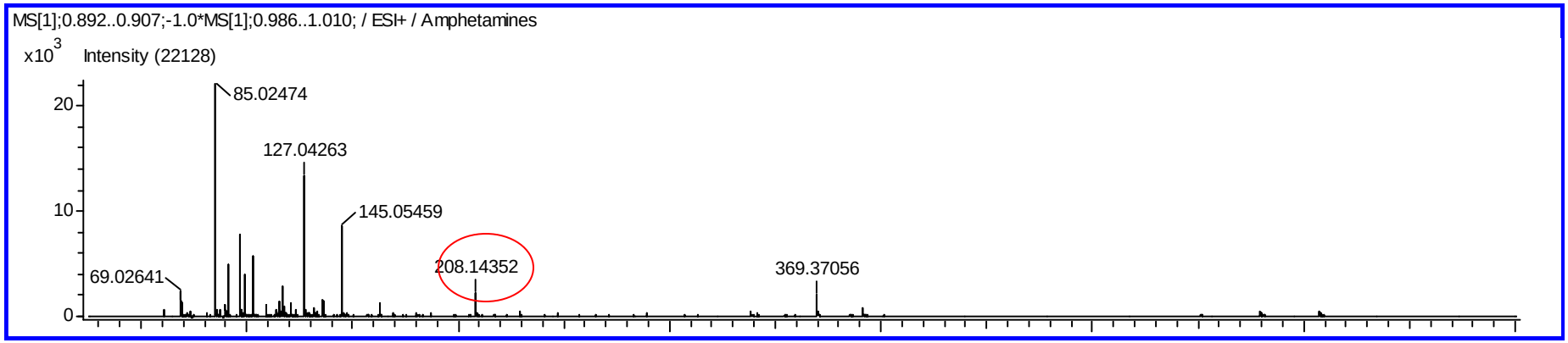


C-8.2. 100 μg/mL Amphetamine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

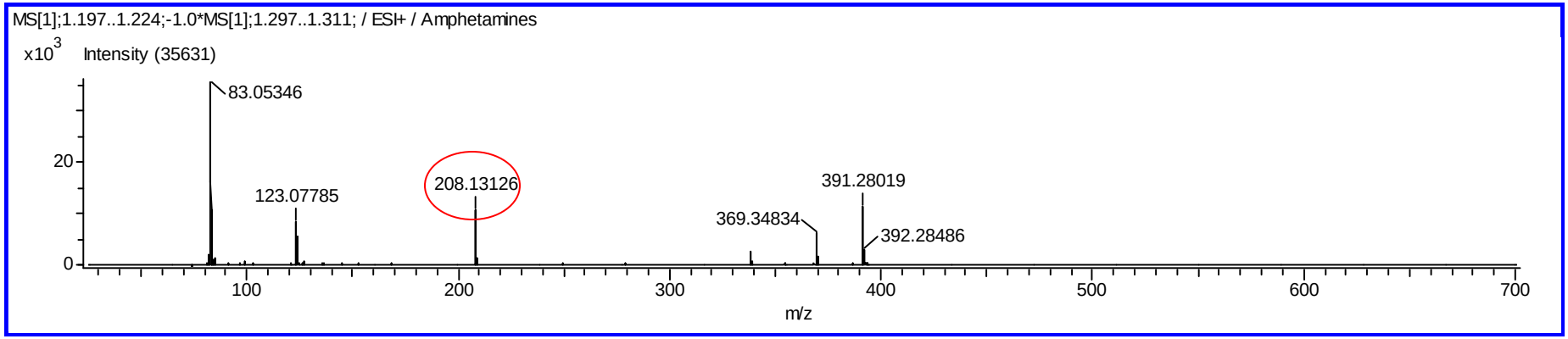


C-8. Amphetamines (continued)

C-8.3. 100 μg/mL MDEA in Blank Blood Extracted 1:3 in ACN

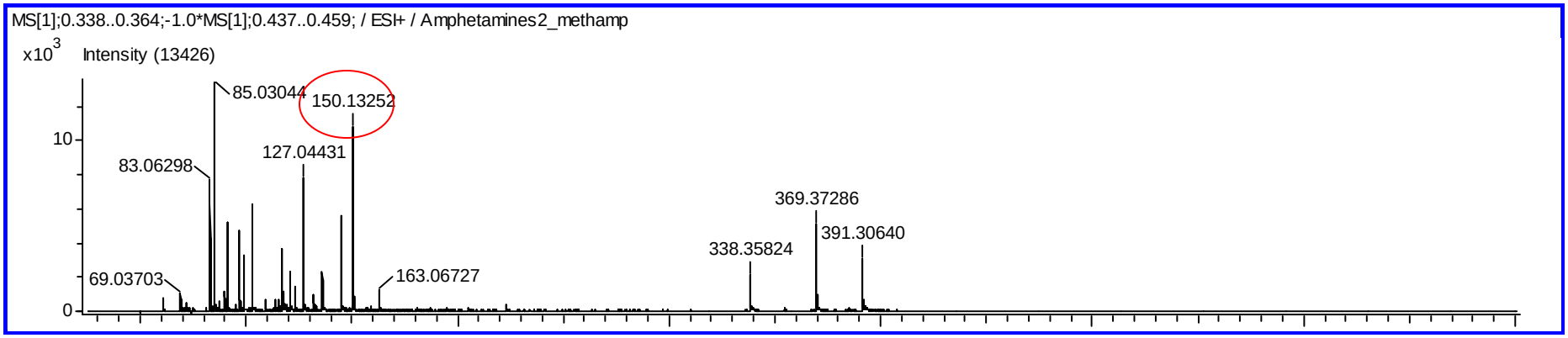


C-8.4. 100 μg/mL MDEA in Blank Blood Extracted in 1:3 ACN/dried/concentrated

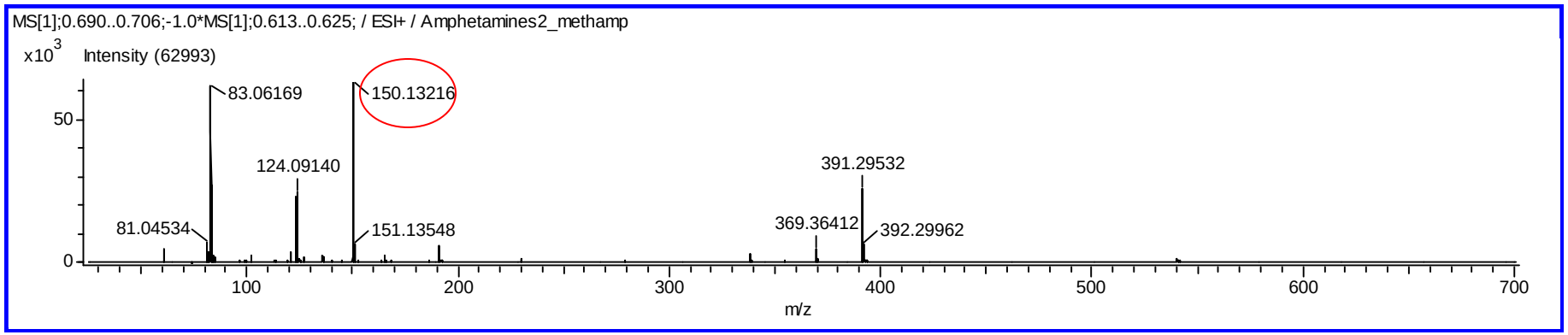


C-8. Amphetamines (continued)

C-8.5. 100 μg/mL Methamphetamine in Blank Blood Extracted 1:3 in ACN

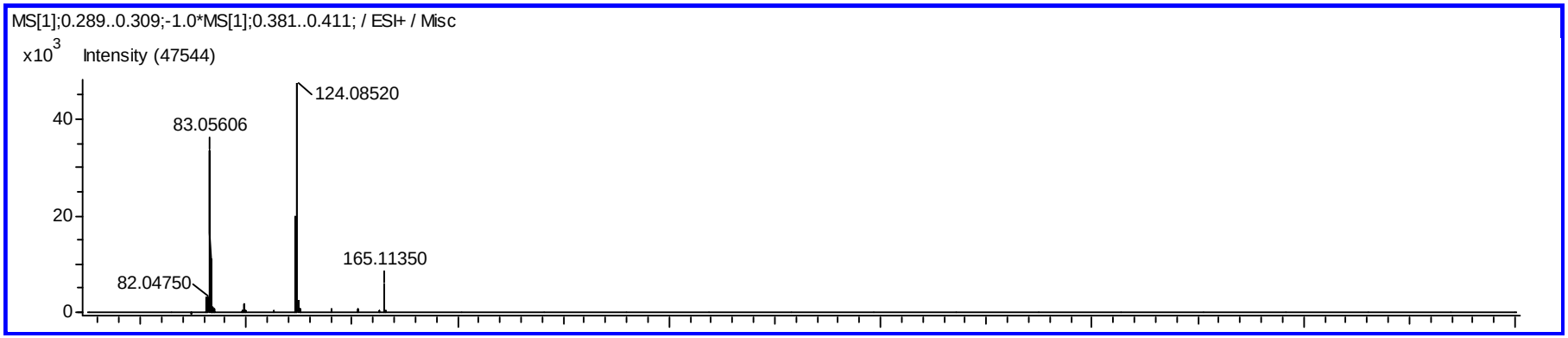


C-8.6. 100 μg/mL Methamphetamine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

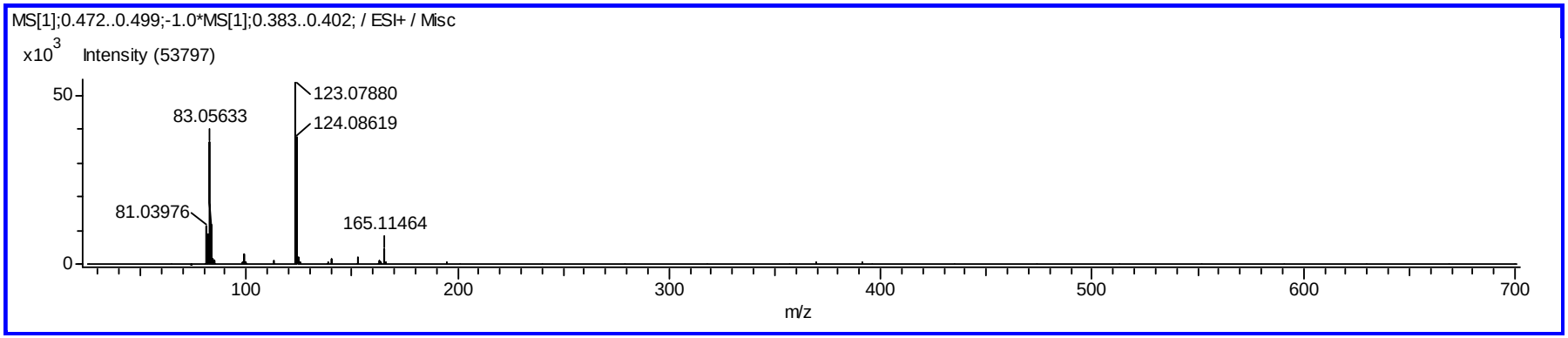


C-9. Miscellaneous

C-9.1. 100 μg/mL Caffeine in Blank Blood Extracted 1:3 in ACN*



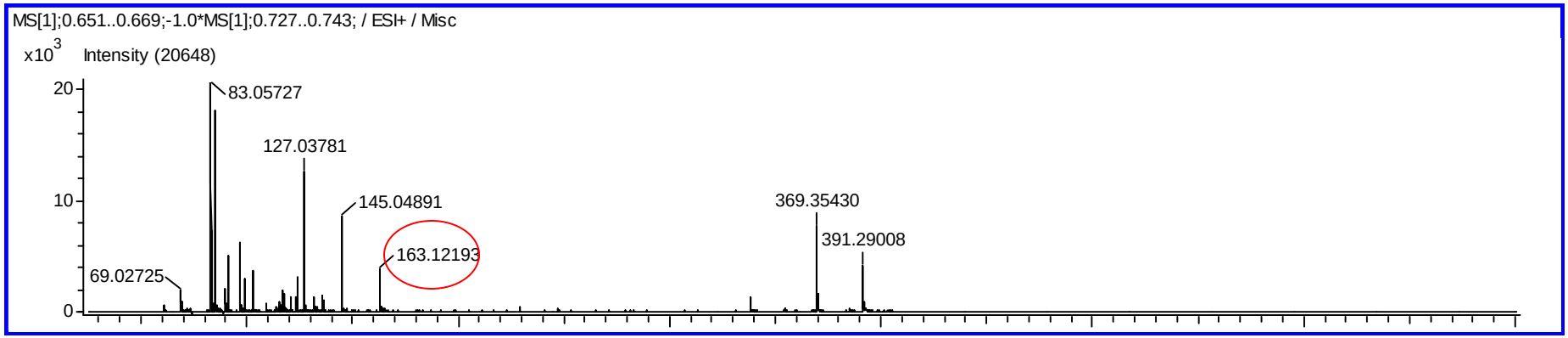
C-9.2. 100 μg/mL Caffeine in Blank Blood Extracted in 1:3 ACN/dried/concentrated*



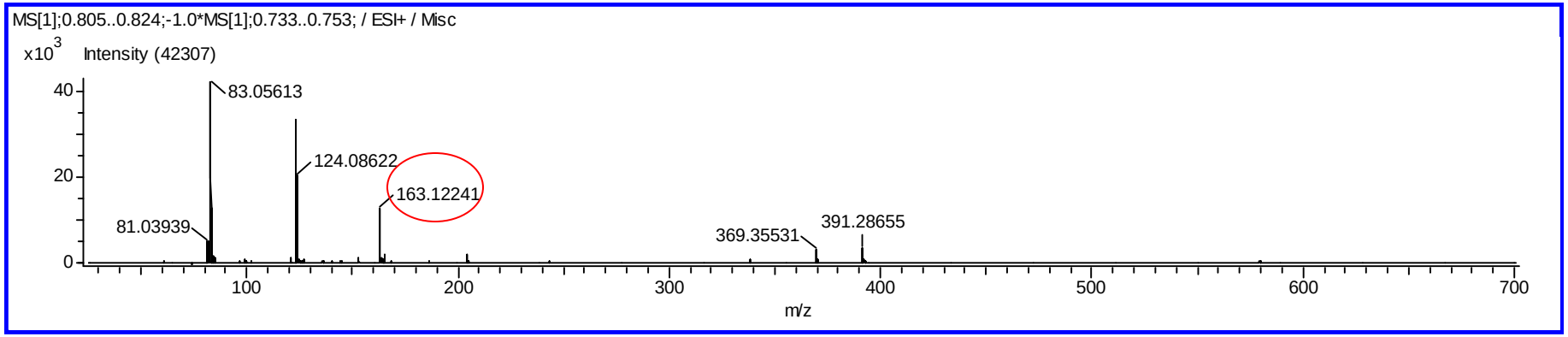
*Not detected

C-9. Miscellaneous (continued)

C-9.3. 100 μg/mL Nicotine in Blank Blood Extracted 1:3 in ACN

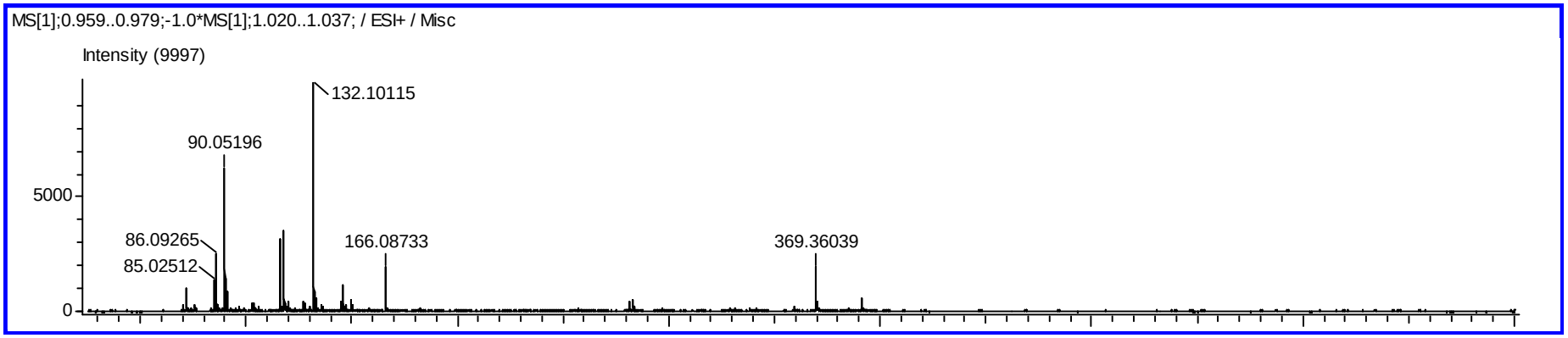


C-9.4. 100 μg/mL Nicotine in Blank Blood Extracted in 1:3 ACN/dried/concentrated

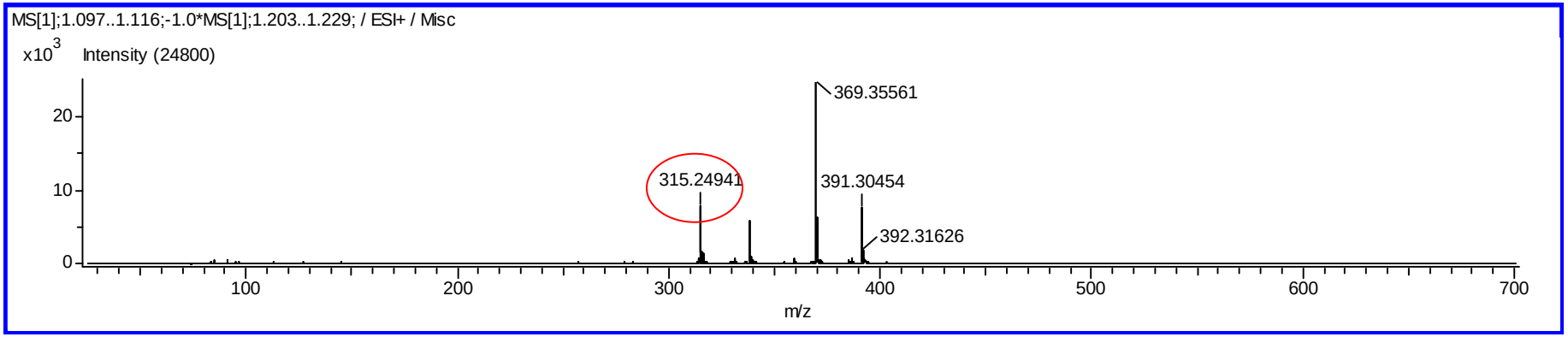


C-9. Miscellaneous (continued)

C-9.5. 100 μg/mL Δ6-THC in Blank Blood Extracted 1:3 in ACN*



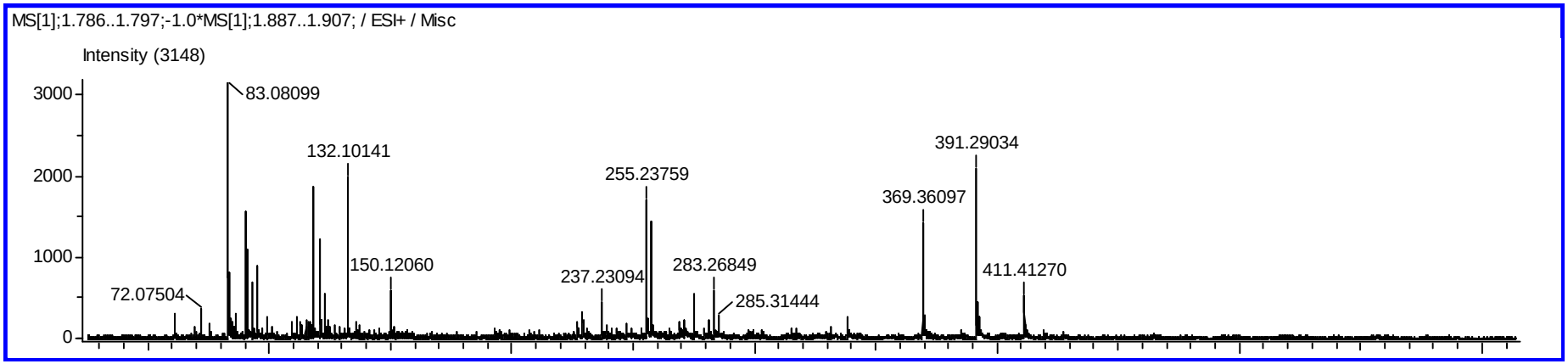
C-9.6. 100 μg/mL Δ6-THC in Blank Blood Extracted in 1:3 ACN/dried/concentrated



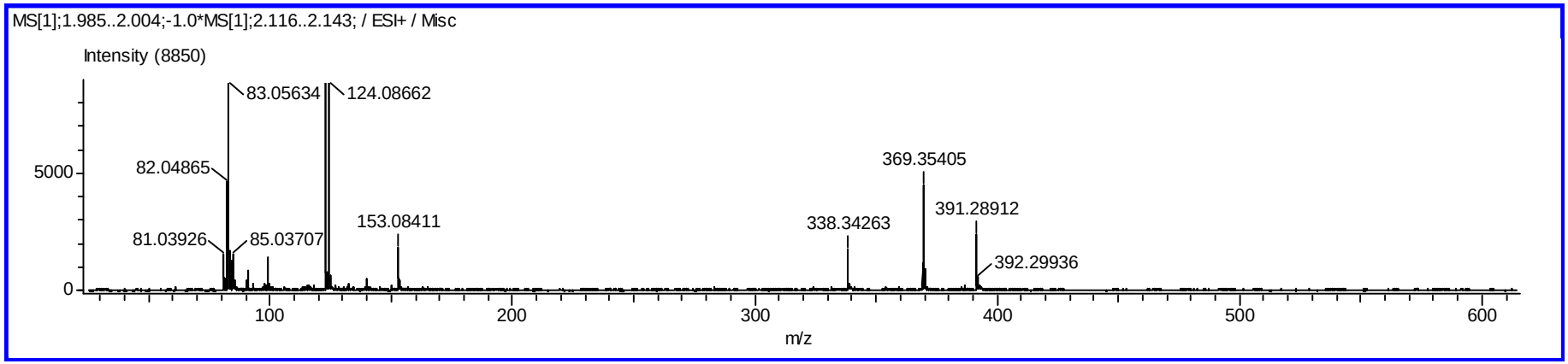
*Not detected

C-9. Miscellaneous (continued)

C-9.7. 100 μg/mL LSD in Blank Blood Extracted 1:3 in ACN*



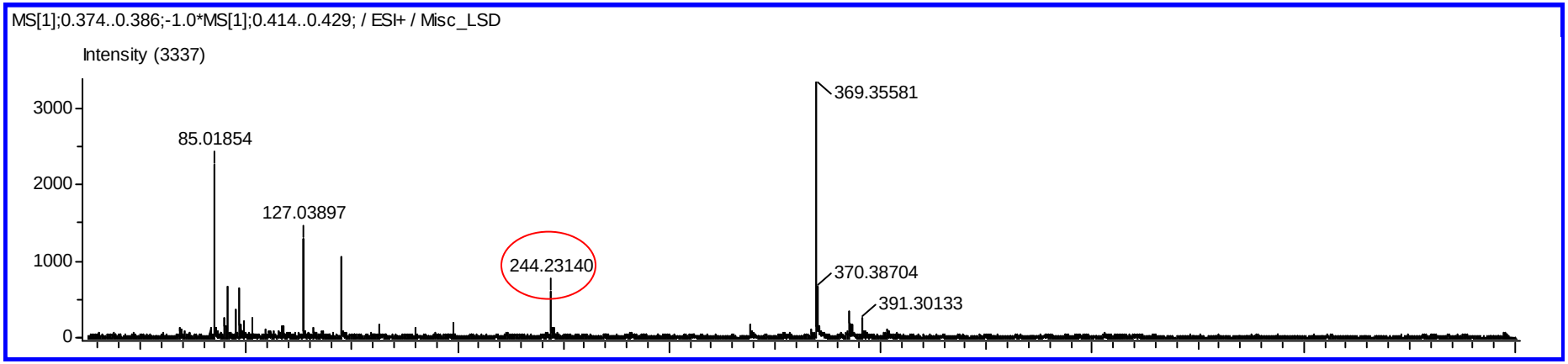
C-9.8. 100 μg/mL LDS in Blank Blood Extracted in 1:3 ACN/dried/concentrated*



*Not detected

C-9. Miscellaneous (continued)

C-9.9. 100 μg/mL PCP in Blank Blood Extracted 1:3 in ACN



C-9.10. 100 μg/mL PCP in Blank Blood Extracted in 1:3 ACN/dried/concentrated