Training Procedure for Gas Chromatography-Mass Spectrometry

1.0 Purpose - The beginning of Mass Spectrometry (MS) date to before 1920, but mass spectrometers were not produced commercially until around 1940. Today the mass spectrometer is one of the most widely used instruments in analytical chemistry. It has both qualitative and quantitative applications in Forensic Toxicology. There are different types of mass spectrometers in use. Ion Trap Mass Spectrometers, Time of Flight Mass Spectrometers (TOF), Quadrupole Mass Spectrometers, Laser Ionization Mass Spectrometers, Chemical Ionization Mass Spectrometers, and Tandem Mass Spectrometers are among many examples. This training unit will familiarize the Forensic Scientist with Gas Chromatograph-Mass Spectrometer (GC/MS) basics and the hardware configuration of the Electron Impact (EI) Mass Selective Detector (MSD) through the answer of study questions. The trainee shall gain knowledge of the operation and maintenance of the GC-MS(s) used in the Toxicology Section through experimental/practical exercises, and shall use the GC-MS to identify a set of unknown substances.

2.0 Scope - This procedure applies to trainees in the Toxicology Section of the State Crime Laboratory.

3.0 Procedure

3.1 Objectives

3.1.1 Review and understand the Toxicology Section technical procedure Toxicology Gas Chromatography-Mass Spectrometry (GC-MS).

3.1.2 Become familiar with the components of the GC-MS.

3.1.3 Understand MS theory and concepts.

3.1.4 Gain practical knowledge of the operation of the GC-MS.

3.1.5 Use the GC-MS to identify drugs and their metabolites.

3.1.6 Successfully complete a written exam with a minimum score of 85 %.

3.2 Study Questions

3.2.1 Describe the components of a GC-MS system.

3.2.2 What is the difficulty in interfacing a GC with a MS?

3.2.3 Name the three major functional components of the MS, and describe how each function.

3.2.4 Explain the term “mean free path.” How is this achieved in a mass spectrometer?

3.2.5 Define the term “base peak” with respect to a mass spectrum.

3.2.6 Define the term “molecular ion” with respect to a mass spectrum.

3.2.7 Explain the term mass defect.

3.2.8 What does tuning the mass spectrometer do?

3.2.9 What is the difference between full scan and selected ion monitoring (SIM) mode?

3.2.10 Explain the phenomenon of "spectral tilting."

3.2.11 Most MS systems have sophisticated search algorithms which perform mass spectral searches of unknown mass spectra. No search routine can provide conclusive identification 100 % of the time. Interpretation and identification is the responsibility of the analyzing scientist. What are some factors that would affect a library search?

3.2.12 Explain the "Nitrogen Rule."

3.2.13 Explain McLafferty rearrangement.

3.2.14 Define the terms nominal mass and resolving power, and explain the concept of resolution in mass spectrometry.

3.2.15 Describe how decomposition can occur in GC-MS analysis. Give examples.

3.2.16 What is derivatization? When would it be useful in GC-MS? List common derivatizing agents.

3.2.17 What functional groups are typically derivatized by acetic anhydride and BSTFA with 1 % TMCS? List three drugs where derivatization by BSTFA with 1 % TMCS may be useful in GC-MS identification.

3.3 Qualitative Evaluation of Mass Spectral Data Using Agilent ChemStation software

3.3.1 Background: Data generated from a GC-MS analysis are stored as an array of mass spectra collected over the time of a GC analysis. For a typical toxicology GC-MS analysis at the NC State Crime Lab, there are about 4000 mass spectra collected during the analysis of a single sample. When a chromatographic peak elutes from the GC there may be several mass spectra collected while the compound is eluting, with the most intense one at the apex of the peak. Even with extraction or clean-up of the sample, biological samples can yield very complex chromatographic mixtures. This block of training will present some tools to use in the analysis of the GC-MS data from these samples.

3.3.2 Definitions

3.3.2.1 Internal Standard – Analyte(s) of similar properties to analytes being extracted. The internal standard is added in known concentration to all samples to evaluate efficiency of extraction and instrumentation in an analytical run. It provides a time basis for relative retention time calculations. The analyte(s) chosen for an internal standard should not be a drug encountered in casework.

3.3.2.2 Total Ion Chromatogram (TIC) - A sample's chromatograph based on the sum of ion abundances in individually collected mass spectra over time.

3.3.2.3 Extracted Ion Chromatogram (EIC) – A sample's chromatograph based on the abundance of a single ion from individual mass spectra over time.

3.3.2.4 Library search – The comparison of an unknown mass spectrum to a library collection of mass spectra of drug standards. This is automated by software that generates a number reflecting the level of similarity between the unknown spectrum and the most similar spectrum in the library.

3.3.2.5 Mass Spectrum – A graph of intensity (abundance) vs. the mass-to-charge ratio (m/z) of an ionized molecule or atom. It represents the distribution of the masses of an ionized molecule and/or its fragments.

3.3.2.6 Blank – A sample which only consists of the reconstitution solvent used in the extraction. The blank identifies potential carryover.

3.3.2.7 Negative Control – An analyte free sample of the same matrix (e.g., blood or urine) as the samples being analyzed. It is prepared and analyzed in the same manner as the samples being analyzed.

3.3.2.8 Positive Control – A sample of the same matrix (e.g., blood or urine) as the samples being analyzed, which is spiked with known analytes prior to extraction. Prepared and analyzed in the same manner as the samples being analyzed. Demonstrates extraction and instrumental acceptance.

3.3.2.9 Probability Based Matching (PBM) – A statistical comparison function used to generate a numerical representation of the similarity of two spectra. This is typically used to determine the best match in automated library searches.

3.3.3 GC-MS Data Evaluation tools (order of use is non-specific, except that subtraction is typically done in response to the use of the other tools)

3.3.3.1 Negative Overlay

3.3.3.1.1 A negative overlay is the combined presentation of the chromatograms (either EIC or TIC) of a sample and a negative control for direct visual comparison.

3.3.3.1.2 This tool can be used only when the negative control and the sample are the same matrix and have been both processed and analyzed in the same manner.

3.3.3.1.3 When overlaying the negative control and the sample, some peaks will appear at the same retention time, have the same shape, and relative intensity in both samples. These peaks are indicative of substances that are either common to the matrix (e.g., fats or cholesterols) or have been introduced to the samples from the chemicals and supplies used in the procedure (e.g., plasticizers, oils).

3.3.3.1.4 A TIC negative control overlay is used to exclude peaks common to both the negative control and the sample from closer examination, unless there are other indications of a drug's presence. Typically, a negative overlay is done and peaks appearing in the sample, but not in the negative control, are library searched.

3.3.3.1.5 Negative control overlays may also be performed to subtract co-elution from an analyte.

3.3.3.2 Library Search of GC-MS Data

3.3.3.2.1 When performing a library search report, a preliminary search may be performed using the NIST library, the most comprehensive library used in the Toxicology Section, for indications of substances that may be present.

3.3.3.2.2 When performing a library search for identification, use the following libraries in the order listed:

3.3.3.2.2.1 TOX_MSCERT{latest date}.L with a minimum 20 match quality.

3.3.3.2.2.1.1. Quality match may be lowered based on quality of mass spectra (e.g. Mepivacaine).

3.3.3.2.2.2 The most current SWGDRUG library (e.g., SWGDRUG_20180416.L) with a minimum 20 match quality .

3.3.3.2.2.3 The most current NIST MS library.

3.3.3.2.3 Other libraries approved by Toxicology Technical Leader may also be used.

3.3.3.2.4 The "TOX_MSCERT{latest date}.L" library was created with traceable information so it may be used for reporting purposes.

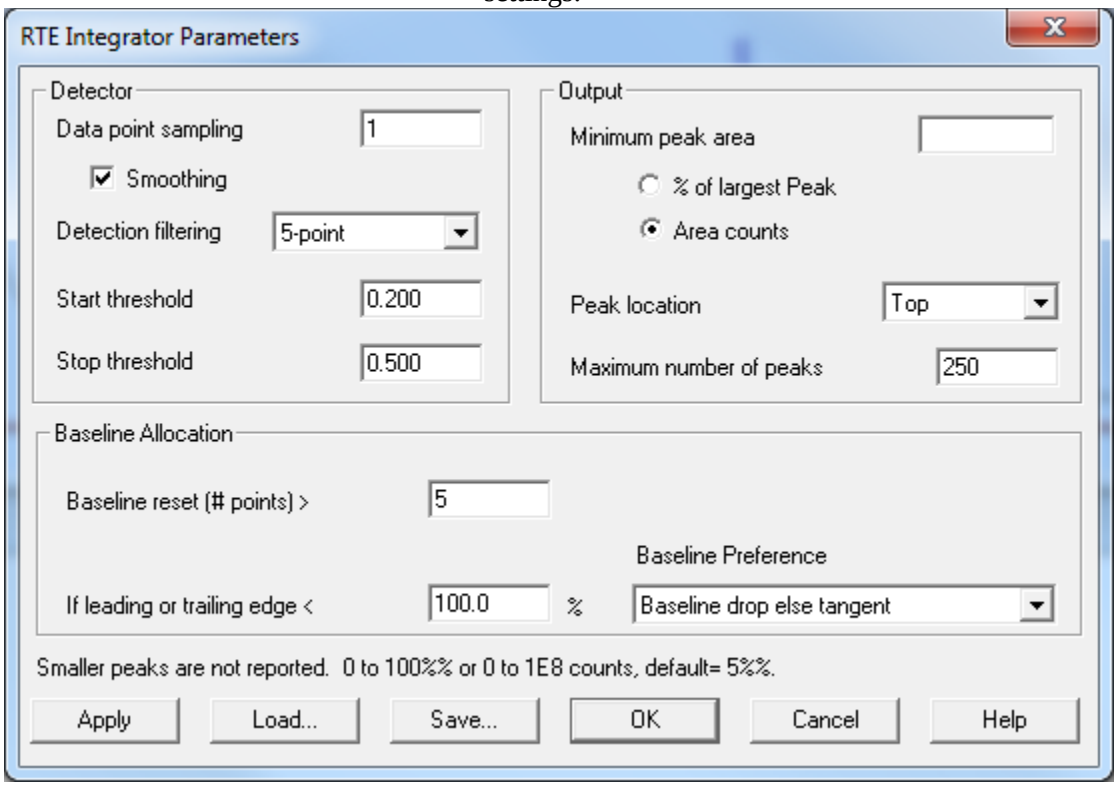
3.3.3.2.5 Each library will be searched in their selected order until a match with quality greater than the minimum selected for the library is found. It is possible that a match with a quality of 20 occurs in the first library, but an entry in the second library would have had a match of 99. The better 99 match will not appear on the library search report since the application would stop with the first library's match.

3.3.3.2.6 When a spectrum contains only a few (e.g., 1-3) intense ions and other ions with intensities less than 10-20 % of the main ions, the PBM results may be less than 40 even though it satisfies the mass spectral identification criteria in the technical procedure for Toxicology Gas Chromatography-Mass Spectrometry.

3.3.3.2.7 When there are several ions with high intensities, PBM results are much less influenced by minor variations in ion intensities.

3.3.3.2.8 The automated Library Search Report is used to generate a report of library searches of the mass spectra in the most significant peaks in a TIC.

3.3.3.2.8.1 Use the RTE integrator's default settings for automated peak integration. Integration is done by the Library Search Report application to identify peaks to be searched against the selected library/libraries. Refer to below for default settings.



3.3.3.2.8.2 To perform a Library Search Report, select "Spectrum" and select "Library Search Report…" Use the Summary style, at the Apex "Spectrum to use."

3.3.3.2.8.3 On the report, evaluate each peak's library search results. The quality of the search results (PBM percent similarity) can be used to identify possible mass spectrum matches.

3.3.3.2.8.4 If multiple matches are identified in a library, only a specified maximum number (e.g., 3) of best matches will be displayed on the report.

3.3.3.2.9 Many compounds listed as matches are not drugs (e.g., fats, plasticizers, cholesterol, oils, etc.) and can be dismissed.

3.3.3.2.10 If a known impairing substance is listed by a library search (with any PBM quality), the analyst should further evaluate the peak's spectra.

3.3.3.3 In the Total Ion Chromatogram (TIC), zoom in on the peaks within the TIC to determine if co-elutions are present. Co-elutions may be seen as split peaks or shoulders. Co-elutions will require further evaluation.

3.3.3.4 EIC search

3.3.3.4.1 The intensity of one or more ions can be charted versus the retention time.

3.3.3.4.2 In ChemStation software use the menu "Chromatogram – Extracted Ion Chromatograms…"

3.3.3.4.3 By selecting ions characteristic to a compound of interest, the presence of that compound can be indicated or contraindicated. This is possible even in the presence of a large co-elution or in a complex TIC.

3.3.3.4.3.1 If the compound of interest is present, the chromatographic peaks of the selected characteristic ions will line up at the same retention time, which is expected for the compound. Sometimes minor retention time differences in the lined-up peaks will occur. This can be due to spectral tilting.

3.3.3.4.3.2 If a compound is indicated by EIC, further evaluation for that compound will be done.

3.3.3.4.3.3 The lack of any of the selected characteristic ions indicates that the compound is absent or the concentration is below the limit of detection.

3.3.3.5 EIC search using the ChemStation Quantitation (no quantitative data is generated).

3.3.3.5.1 ChemStation's Quantitation application can be used to automate the process of displaying multiple compounds' characteristic ions at their expected retention times.

3.3.3.5.2 The quantitation application uses up to four characteristic ions, their expected intensities relative to each other, and the expected retention time to identify a peak before quantitation is performed. Even without quantitation calibration data, the application will display the results of the peak identification.

3.3.3.5.2.1 Detector responses may change over time, so a compound's ion's relative responses may not be stable over multiday time periods.

3.3.3.5.2.2 Retention times (RT) may change due to maintenance, column age, or different instruments. Relative retention times (RRT) can be used to normalize these differences; however, the ChemStation quantitation application uses the RT for peak identification.

3.3.3.6 Subtraction of Mass Spectrum

3.3.3.6.1 Definition of Mass Spectrum Subtraction - The subtraction of each m/z's intensity in one mass spectrum from that of another mass spectrum.

3.3.3.6.2 In ChemStation, subtraction is done by selecting the mass spectrum of interest; then selecting the mass spectrum to be subtracted from the one of interest; then selecting the menu "Spectrum-Subtract."

3.3.3.6.2.1 The selected mass spectra (of interest and to be subtracted) can be an individual mass spectrum or an averaged range of mass spectra.

3.3.3.6.3 The spectrum at the apex of the peak will be used to represent the peak of interest, when no co-elutions are present.

3.3.3.6.3.1 When a co-elution is present, overlaying an EIC of an ion(s) from the peak of interest and an ion(s) from the co-elution can help identify the part of the peak that gives the best response relative to the co-elution.

3.3.3.6.4 When selecting a spectrum to subtract, chromatographic noise prior to the peak of interest is typically selected. Spectra from peaks co-eluting with the peak of interest (before or after the peak) can be selected.

3.3.3.7 Retention time evaluation

3.3.3.7.1 To determine the relative retention time of an analyte, divide the retention time of the analyte at the most abundant point of the analyte by the retention time of the internal standard at the internal standard's most abundant retention time.

3.3.3.7.2 For identification, an evaluation must be made of the analyte's relative retention time difference as compared to the relative retention time of the certified reference material for that analyte. The difference must be less than 2% for identification.

3.3.3.7.2.1 RRT Difference Calculation:
100 * (standard RRT-analyte RRT)/ (standard RRT)

3.4 Practical/Laboratory Exercises

3.4.1 Tune a MS. Compare the tune report for the MSD with the requirements stated in the Toxicology Section Toxicology Gas Chromatography-Mass Spectrometry procedure. What is the significance of each tune requirement?

3.4.2 Observe the Toxicology Training Coordinator or designee perform routine maintenance including syringe check and liner change followed by the run and analysis of the performance check.

3.4.3 Observe the Toxicology Training Coordinator or designee prepare to use a GC-MS, setup a sequence, run a sequence and analyze data files. Using the GC-MS, review the data files provided by the Toxicology Training Coordinator. The data files consist of sets of substances that produce similar mass spectra. Attempt to group the substances into pairs and identify each substance. Describe the criteria used to differentiate between the substances.

3.4.4 Review the mass spectra for dextromethorphan and dextropropoxyphene. Is it possible to identify optical isomers using mass spectral data?

3.4.5 What change would occur in the TIC and the MS if the electron multiplier voltage were increased? The standard energy for the beam of ionizing electrons in EI MS is 70 eV. What would be the effect if the voltage of the ionizing source were changed?

3.4.6 Review with a senior analyst and demonstrate the use of GC-MS data analysis software parametric retrieval to search a mass spectral library and view mass spectra in the library.

3.4.7 Review with a senior analyst and demonstrate the use of GC-MS data analysis software to determine the signal to noise ratio (S/N) of a chromatographic peak.

3.4.8 Process positive and negative control samples provided by the trainer and evaluate their GC-MS data as required by the current extraction and GC-MS technical procedures.

3.4.9 Propose molecular structures for m/z ions in the following mass spectra, and answer the questions.

3.4.9.1 Blank /baseline – 44. What is the origin of the 207 m/z ion?

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File       : D:\DATA\OCTOBER2006\A002014.D
Operator   : Serial # US21863672
Acquired   : 16 Oct 2006  17:50     using AcqMethod 20HIGH
Instrument :    US2186367
Sample Name: MeOH
Misc Info  :
Vial Number: 100
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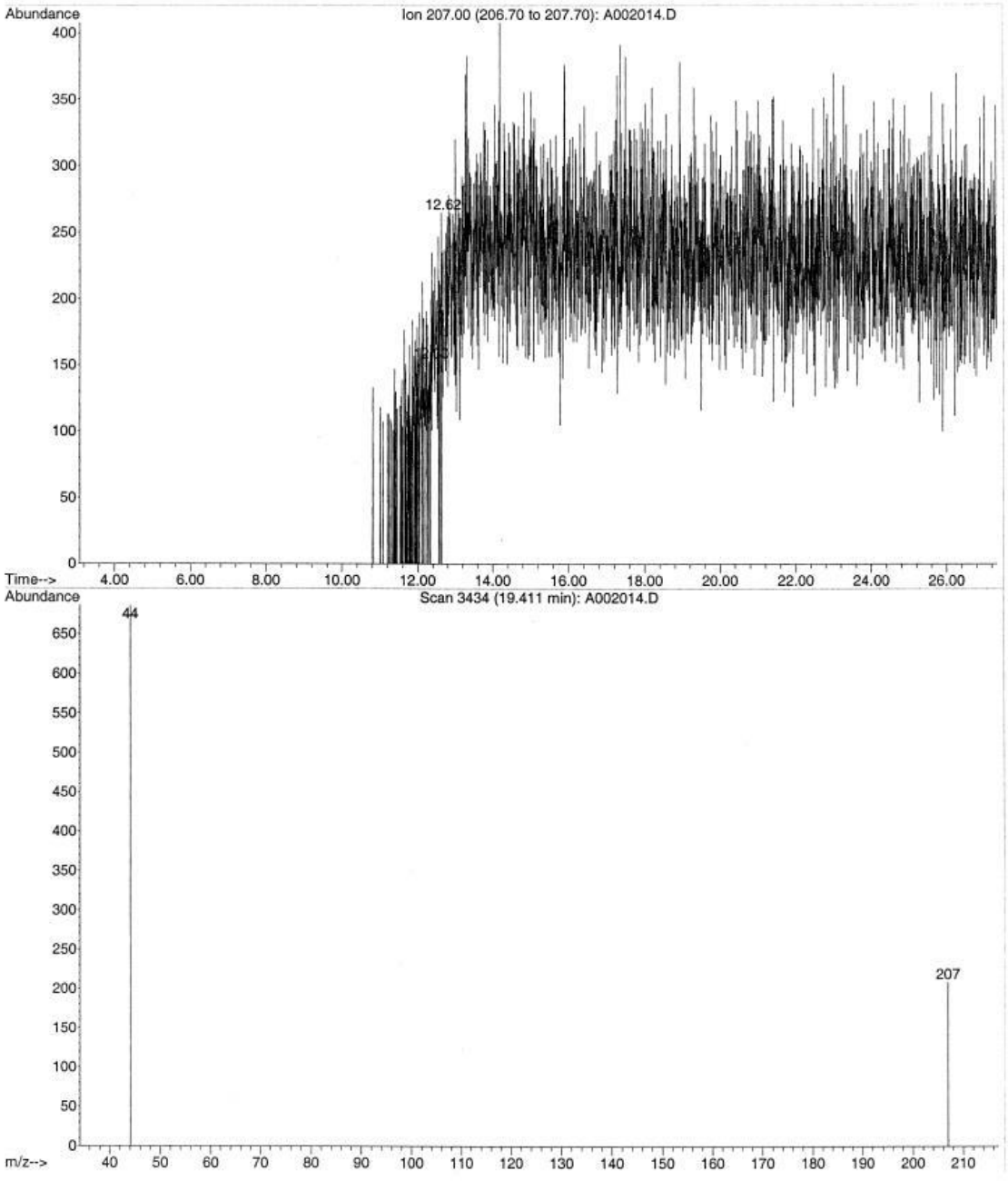
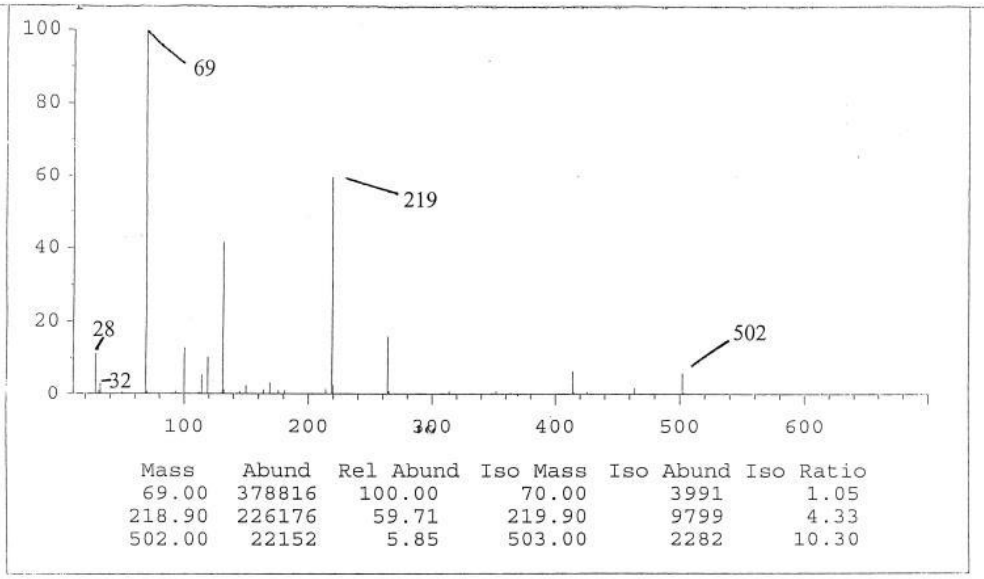


3.4.9.2 PFTBA - 69, 219, 502. The m/z 32 and 28 m/z ions are not ions of PFTBA. What would cause these ions?



Mass	Abund	Rel Abund	Iso Mass	Iso Abund	Iso Ratio
69.00	378816	100.00	70.00	3991	1.05
218.90	226176	59.71	219.90	9799	4.33
502.00	22152	5.85	503.00	2282	10.30

Perfluorotributylamine (PFTBA or FC_{43})

3.4.9.3 Cocaine - 105, 182

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Cocaine (SIGMA C-5776 Lot 043K1284)

Entry Number          22          from    C:\Database\CERTDRUGS.L
CAS                   000050-36-2
Melting Point         -300
Boiling Point         -300
Retention Index       0
Mol Formula           C17H21NO4
Mol Weight            303.147
Company ID

Miscellaneous Information
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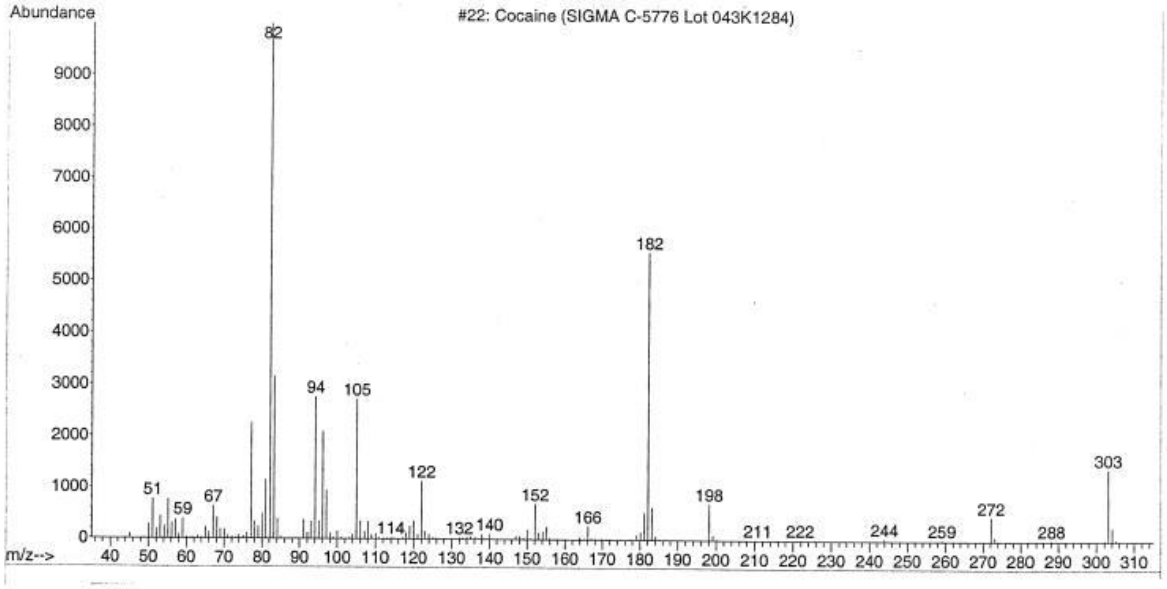


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C:\Database\CERTDRUGS.L          Tue Oct 17 18:33:29 2006          Page 1
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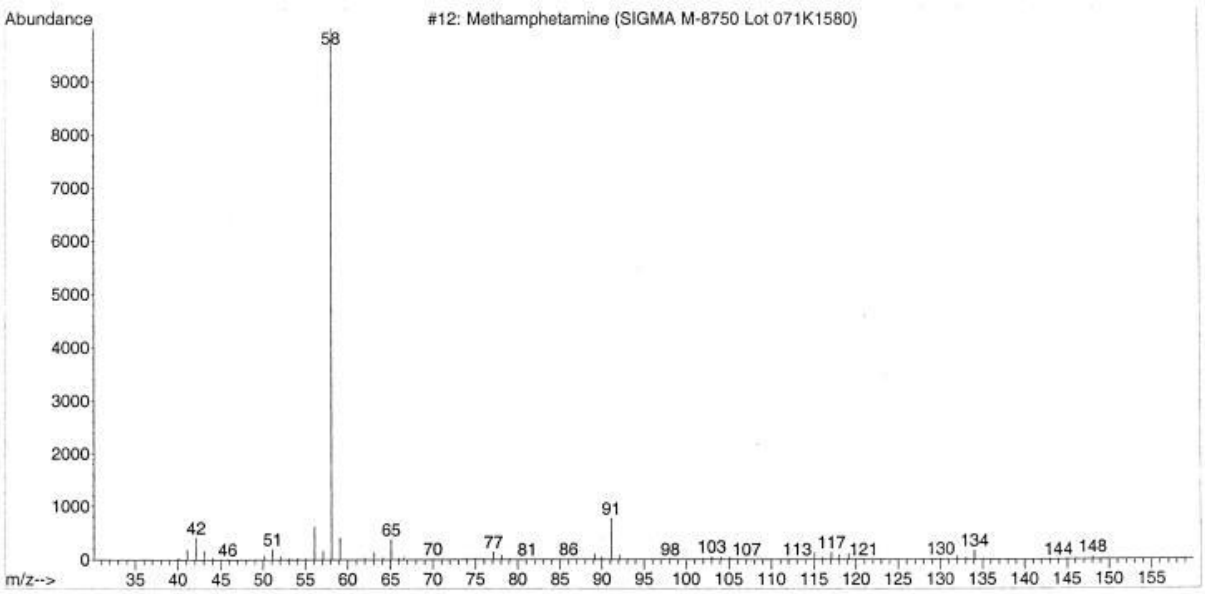
3.4.9.4 Methamphetamine - 58, 91. What is the name of the 91 m/z ion?

Methamphetamine (SIGMA M-8750 Lot 071K1580)

Entry Number	12	from	C:\Database\CERTDRUGS.L
CAS	000537-46-2		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C10H15N		
Mol Weight	149.12		
Company ID			

Miscellaneous Information



No structure available for 000537-46-2

C:\Database\CERTDRUGS.L Fri Nov 03 09:37:54 2006 Page 1

3.4.9.5 Amphetamine – 44, 91

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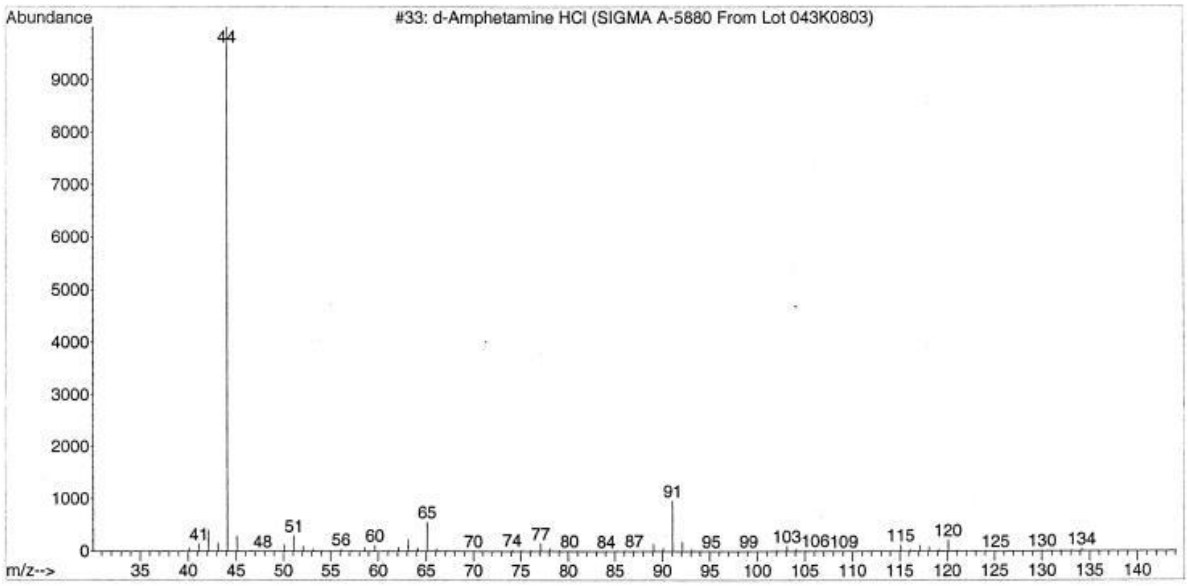
d-Amphetamine HCl (SIGMA A-5880 From Lot 043K0803)

Entry Number          33          from    C:\Database\CERTDRUGS.L
CAS                   000000-00-0
Melting Point         -300
Boiling Point         -300
Retention Index       0
Mol Formula           C18H28N2
Mol Weight            272.23
Company ID

Miscellaneous Information
   Acid extract of Amphetamine Sulfate purchased standard.
```



No structure available for 000000-00-0

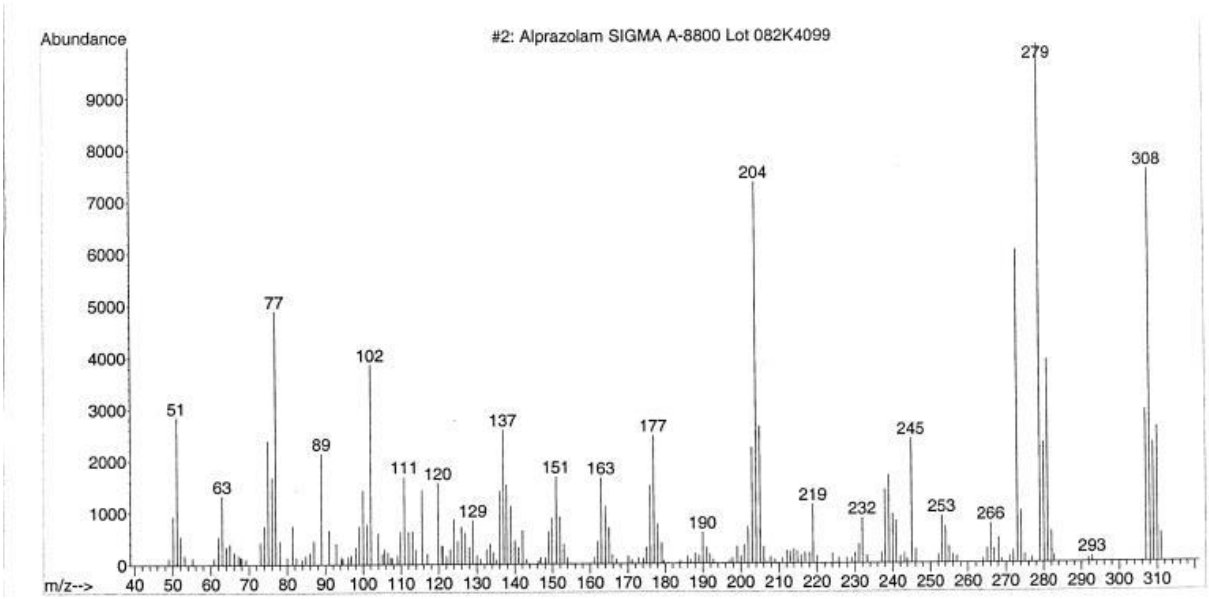
C:\Database\CERTDRUGS.L Mon Oct 30 16:34:37 2006 Page 1

3.4.9.6 Alprazolam – 77. Explain the significance of the abundances in the 279 / 281 m/z pair and the 308 / 310 m/z pair.

Alprazolam SIGMA A-8800 Lot 082K4099

Entry Number	2	from	C:\Database\CERTDRUGS.L
CAS	028981-97-7		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C17H13ClN4		
Mol Weight	308.083		
Company ID			

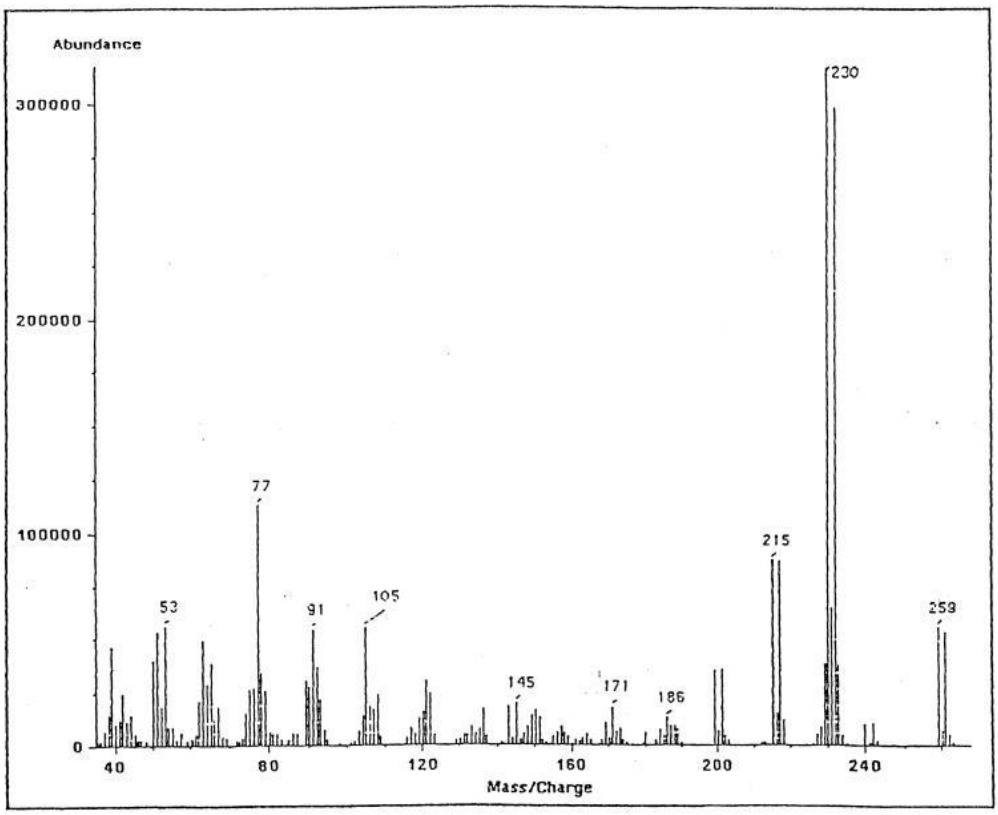
Miscellaneous Information



No structure available for 028981-97-7

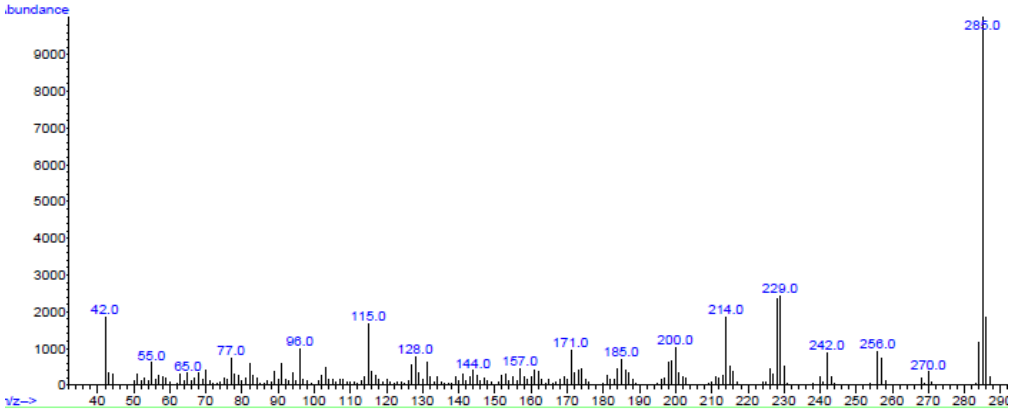
C:\Database\CERTDRUGS.L Tue Oct 17 18:32:46 2006 Page 1

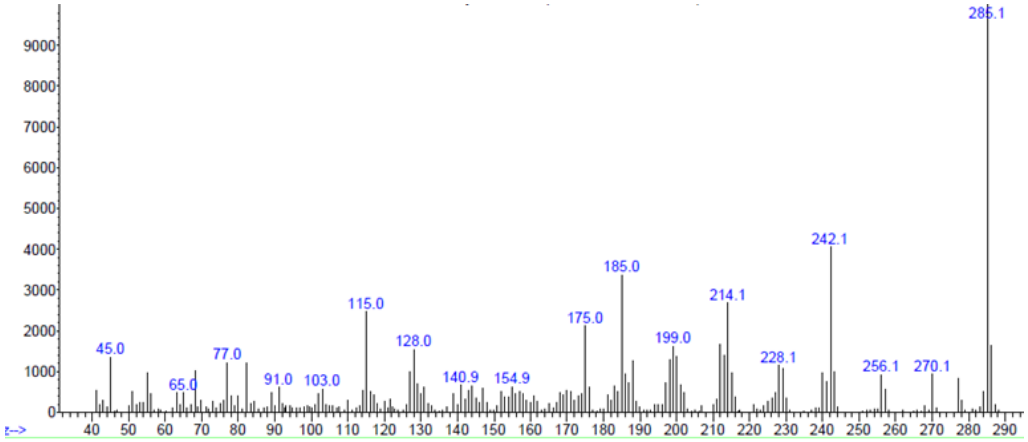
3.4.9.7 4-bromo-2, 5-dimethoxypenethylamine - 215.
Explain the significance of the abundances in the 215 / 217 m/z, 230 / 232 m/z, and the 259 / 261 m/z pairs.



4-bromo-2,5-dimethoxyphenethylamine

3.4.9.8 What are the following two opiates and how is an evaluation made between them?



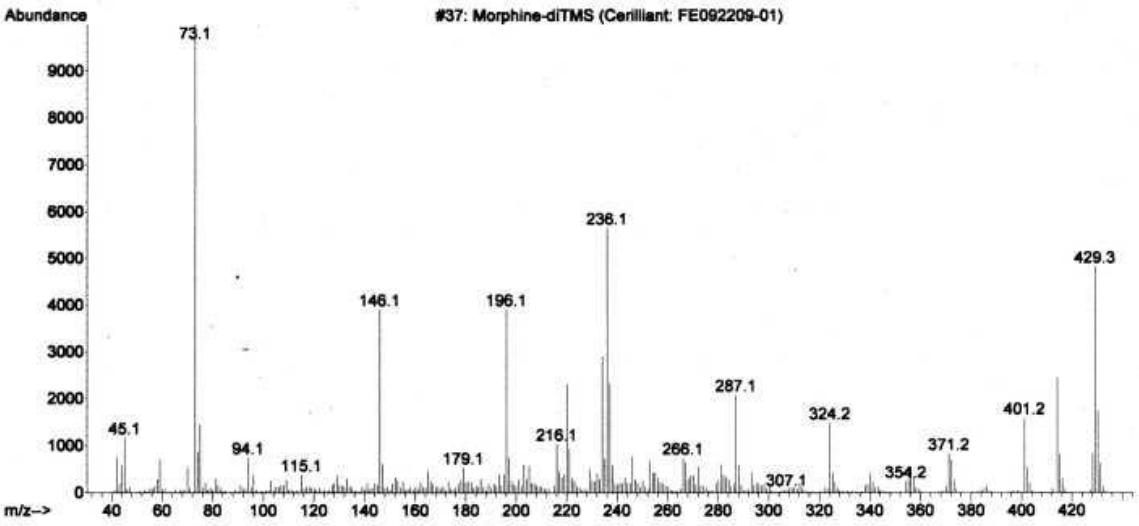


3.4.9.9 Morphine-diTMS – 73, 429

Morphine-diTMS (Cerilliant: FE092209-01)

Entry Number	37	from	C:\msdchem\1\Library\TOX_MSCERT20140516DRAFT UPDATES.L
CAS	055449-66-6		
Melting Point	-300		
Boiling Point	-300		
Retention Index	0		
Mol Formula	C23H35NO3Si2		
Mol Weight	429.215		
Company ID			

Miscellaneous Information
NIST:Morphinan,7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5a,6a)-



No structure available for 055449-66-6

C:\msdchem\1\Library\TOX_MSCER Fri May 30 08:25:56 2014 Page 1

3.5 Required Reading

Agilent GC-MSD ChemStation and Instrument Operation Student Manual Course Number H4043A Volume 1, Revision E.02.xx, Agilent Technologies: printed February 2008, pp 1-32, 39-73, 81-122.

Toxicology Gas Chromatograph-Mass Spectrometry procedure

GCMS Data Processing procedure

4.0 References

Hewlett Packard / Agilent Technologies. *GC MSD ChemStation and Instrument Operation Student Manual, Vol. I & II, (Manual Part Number H4043-90000)*. Hewlett Packard: April, 1997.

F.W. McLafferty. *Interpretation of Mass Spectra*. 2nd Ed. W.A. Benjamin and Sons: 1973.

BSTFA with 1 % TMCS Product Specification. Sigma-Aldrich Co.: 1997.

Guide to Derivatization Reagents for GC, Bulletin 909A. Sigma-Aldrich Co.: 1997.

Derivatization of Drugs Prior To GC-MS Analysis. Varian Application Note Number 69. Varian Inc.

http://www.chem.agilent.com

5.0 Records

Toxicology Drug Training Checklist

Training Section Completion Summary

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
05/10/2013	2	3.1.6 - inserted criteria for successful completion and removed "on chromatography" for exam descriptor
11/15/2013	3	Added issuing authority to header
06/13/2014	4	In the title and purpose (1.0) clarified that the training related to GC/MS 3.2.17 – added newly incorporated derivatizing agent Added 3.3 3.4 – changed "m/e" to "m/z" to be consistent with current literature 3.4.4 – clarified the question Added 3.4.6, 3.4.7, 3.4.8, 3.4.9 3.4.10 - reduced file size of mass spectrum pictures 3.4.10.6 (3.3.6.5) and 3.4.10.7 (3.3.6.6) – clarified the question 3.4.10.8 (3.3.6.7) – replaced procaine example with morphine-diTMS 3.4.10.9 (3.3.6.8) – removed question
02/22/2019	5	Replaced references to Drug Chemistry Section/Toxicology Unit with Toxicology Section 3.1.5 Replaced "controlled substances" with "drugs" and their metabolites 3.2 Replaced all "MSD" references with "MS" 3.2.16 Removed "and their applications" 3.3.1 Corrected "3,400" with "4,000" 3.3.2 Add definitions for Internal Standard, Blank and Positive Control 3.3.2.7 – Add "analyte free" Added: 3.3.3.1.5 3.3.3.2.1: Added that a preliminary search may be done using NIST 3.3.3.2.2 – Added "When performing a library search for identification" 3.3.3.2.2.1 and 3.3.3.2.2.2 – Replaced "40" with "20" for minimum match quality Added: 3.3.3.2.2.1.1 3.3.3.2.2.2 – Replaced AAFS library with SWGDRUG library 3.3.3.2.2.3- Removed MPW as a library to be used when performing a library search and replaced with AAFS 3.3.3.2.3 – Added "approved by Toxicology Technical Leader" 3.3.3.2.5 – Replaced "40" with "20" in referencing a library search. 3.3.3.2.8.1 – Give default settings for RTE integrator 3.3.3.2.8.2 – Add "Select "Spectrum" and select "Library Search Report…" Added: 3.3.3.3; 3.3.3.7; 3.4.2; and 3.4.9.8. 3.5 – Added GCMS Data Processing Procedure