Gas Chromatography – Mass Spectrometry (GC-MS) Data Processing

1.0 Purpose - This procedure specifies the required elements for processing and acceptability of Toxicology GC-MS data.

2.0 Scope – This procedure applies to Toxicology in the Raleigh, Triad, and Western locations of the State Crime Laboratory.

3.0 Definitions – See Toxicology Definitions List

4.0 Equipment

- PC with Agilent MassHunter and Chemstation software or equivalent, printer or other output device

5.0 Procedure

5.1 Data Processing

5.1.1 All case and control sample data files shall be processed and evaluated in accordance with the acceptance criteria listed below.

5.1.2 All solvent blank samples analyzed immediately prior to case and control samples shall be processed and evaluated in accordance with the acceptance criteria listed in **5.2.3**.

5.1.2.1 If an analyte detected in the solvent blank meets the acceptance criteria listed in **5.2.3**, the analyte shall be evaluated following the procedure outlined in **5.6**.

5.2 Identification for Qualitative Methods

5.2.1 The GC-MS provides retention time data and mass spectral data.

5.2.2 Chromatographic Acceptance Criteria

5.2.2.1 The root-mean-square (RMS) signal to noise ratio of the internal standard(s) must be greater than 5:1. The mass spectral processing software application calculates the RMS signal to noise ratio once the internal standard peak is selected and the baseline or valley immediately before, or after, the internal standard signal is selected.

5.2.2.2 The relative retention time compared to a reference material standard shall have a difference of 2.0 % or less. The standard relative retention time shall be determined under the same chromatographic conditions as the sample.

5.2.3 Mass Spectral Acceptance Criteria

5.2.3.1 The mass spectrum shall be searched and compared to a reference collection of reference material mass spectra. Probability Based Matching (PBM) shall be used to aid the analyst in the identification, but shall not be used as the sole basis of the identification.

5.2.3.1.1 Only mass spectral libraries approved by the Toxicology Technical Leader shall be used to process data.

5.2.3.2 The presence of additional ions in the mass spectrum may be indicative of background noise or a co-eluting substance. **An** attempt should be made to isolate the source of the additional ions and subtract prior to searching the reference collection of reference material mass spectra.

5.2.3.3 The mass spectrum must contain all of the major and diagnostic ions unique to the analyte at a relative abundance consistent with the reference standard spectrum.

5.2.3.4 All ions with a relative intensity greater than 10 % of the base peak in the reference standard spectrum must be present in the sample spectrum.

5.2.3.4.1 For drugs with no ions greater than 10 % of the base peak, magnify the y-axis of the reference standard spectrum and the sample mass spectrum to aid the analyst in identifying the two next most abundant ions with a *m/z* greater than 100 in each spectra.

5.2.3.4.2 The two ions identified in **5.2.3.4.1** for the reference standard spectrum shall be at a relative abundance consistent with the sample mass spectrum

5.2.3.5 The case record shall contain the mass spectrum of the reference material standard with a Drug Chemistry vault ID or supplier/lot number.

5.3 Positive Control Acceptance Criteria

5.3.1 The internal standard and all positive control analytes must meet the identification criteria in **5.2.2** and **5.2.3**.

5.3.2 If a drug intended to be in a positive control is not identified in the positive control, all cases affected by the control failure shall be re-extracted.

5.3.3 For derivatized positive controls, all compounds that are subject to derivatization must be derivatized.

5.3.3.1 If an analyte subject to derivatization fails to derivatize, the data may still be used with approval from the Toxicology Technical Leader.

5.4 Negative Control Acceptance Criteria

5.4.1 The internal standard must meet the identification criteria in **5.2.2** and **5.2.3**.

5.4.2 Any drug identified in the negative control will not be reported positive from the run. All cases affected by the control failure shall be re-extracted.

5.5 Extracted Ion Chromatograms

5.5.1 An EIC shall be generated and included in the case record for the following:

5.5.1.1 Unresolvable co-eluting substance containing an ion that is greater than 50 % of the abundance of the analyte's base ion and which meets the criteria listed in 5.2.3.4.

5.5.1.2 Uncorrelated Amphetamine, Methamphetamine, and Barbiturate Immunoassay results for any analytes listed with the assays on the Toxicology Reporting index.

5.5.2 An EIC shall be labeled with the reason it was generated.

5.6 Carryover Determination

5.6.1 Carryover is evaluated by comparing the ratio of the abundance of the analyte's base ion in the sample to the solvent blank.

5.6.1.1 Perform an EIC of the analyte's base ion in both the sample and the solvent blank and overlay them.

5.6.1.2 If the base ion in the solvent blank is less than 10 % of the abundance of the base ion in the sample, the carryover has a negligible contribution to the analyte signal in the sample.

5.6.1.3 If the base ion in the solvent blank is greater than or equal to 10 % of the abundance of the base ion in the sample, the data for that analyte may not be used for reporting purposes.

5.7 MS Data File Documentation

5.7.1 The processed data shall contain the following:

5.7.1.1 Total Ion Chromatogram of the sample and corresponding blank labeled with peaks of interest.

5.7.1.1.1 Peaks of interest are defined as any substance for which a RRT has been established or that meets the criteria listed in 5.2.3.3 and 5.2.3.4.

5.7.1.2 The following compounds are routinely encountered in mass spectral data processing and do not need to be processed:

- Nicotine
- Cotinine
- Hydroxycotinine
- Nicotinamide
- Caffeine
- Cholesterols
- Fatty Acids
- Phthalates
- Steroids

5.7.1.3 Subtracted mass spectra of internal standard(s) and peaks of interest with library match.

5.7.1.4 Relative Retention Times (RRTs) and % difference of the peak of interest and corresponding reference standard.

5.7.1.5 Extracted Ion Chromatogram generated in the analysis of the data.

5.8 Quality Control Data Packets

5.8.1 QC data packets shall be created for all case sample analyses performed on a GC-MS.

5.8.2 The quality control data packets shall contain the following:

5.8.2.1 Cover page with FA workstation reference

5.8.2.2 Completed extraction worksheet

5.8.2.3 Verified GC-MS sequence list

5.8.2.4 Signed and approved GC-MS tune

5.8.2.5 GC-MS method

5.8.2.6 Negative Controls and associated blanks

5.8.2.7 Positive Controls and associated blanks

5.8.3 Quality control data packets shall be named with a file name beginning with the type of extraction performed (ANSPE, BSPE, TMS, PHEALLE) followed by the eight digit year/month/day format ending with the instrument name. A suffix may be added to differentiate multiple runs.

5.8.3.1 Example: ANSPE20121016T1-XXX or BSPE20121016T1-XXX

5.8.4 All quality control data packets shall be administratively and technically reviewed prior to use of the associated case data for reporting. The review and approval shall be indicated by signing the summary page.

5.8.5 QC packs shall be stored in the Manage Files section of the associated workstation and approved.

5.9 Standards and Controls – N/A

5.10 Calibrations – N/A

5.11 Maintenance – N/A

5.12 Sampling – N/A

5.13 Calculations

5.13.1 RRT Calculation: (analyte retention time / internal standard retention time) rounded to the nearest thousandth. A retention time may be determined as the elution time at which the mass spectrum was collected or with an integrator in the mass spectral processing software.

5.13.2 RRT Difference Calculation:
|(standard RRT – analyte RRT)| / (standard RRT) * 100

5.14 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 Substances that generally cannot be identified by current State Crime Laboratory analytical procedures are listed in the Toxicology GCMS Reporting Index.

6.2 Mass spectrometry alone cannot differentiate isomers.

7.0 Safety – N/A

8.0 References

Toxicology Unit Technical Procedures:

Toxicology GCMS Reporting Index

Forensic Toxicology Laboratory Guidelines, 2006 version; SOFT / AAFS.

Agilent GC-MSD ChemStation and Instrument Operation Student Manual Course Number H4043A Volume 1, Revision E.02.xx. Agilent Technologies, February 2008.

Pfleger, Maurer, and Weber. *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites.* 2nd. Ed., Vols. 1-3, 1992.

9.0 Records

- Processed MS data file
- Approved QC packet

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
06/19/2020	4	3.0 – moved to Toxicology Definitions List 4.0 – updated to include MassHunter 5.2.2.1 and 5.13.1 – Removed reference to Chemstation 5.1.2 and 5.2.3.2 - reworded 5.2.3.3 and 5.2.3.4.2 – inserted language regarding relative abundance 5.2.3.5 – removed "or other appropriate Drug Chemistry Designation" 5.5.1.2 – removed 5.5.1.3 (New 5.5.1.2) – adjusted requirement due to new instrumentation. 5.7.1.1.1 – added additional criteria 5.7.1.2 – replaced with 5.7.1.2.1 5.8.5 – changed "Managed" to "Manage"