#16
General Unknowns
Analytical Method

1.0.0 Background and Scope

There are times when samples do not fit into a certain category. These procedures are designed to analyze these samples, examples of which are pills, liquid pharmaceuticals, and samples that do not give the expected results with screening tests. Whenever possible, two different tests, and two different sampling events will be employed in confirming the presence of controlled substances. One of the tests must provide structural information, i.e. either MS or FTIR.

2.0.0 Equipment and Reagents

The following pieces of equipment can be used in any combination to identify the analytes of interest.

2.1.0 A GC/MS and appropriate analytical software. Reference GC/MS Analytical Method.
2.2.0 FTIR and appropriate analytical software. Reference GendrugAM section 10.
2.3.0 ACS grade solvents.
2.4.0 pH paper.
2.5.0 n-Tridecane internal standard. In the ratio of 1.3ml tridecane into 1 L chloroform.

3.0.0 GC/MS Sample Preparation and Analysis

This section details the minimum requirements for analysis, any additional testing if necessary may be performed at the analyst discretion.

3.1.0 Solids

3.1.1 Extractions. Using appropriate sampling, extract with methanol, chloroform, or a mixture of methanol and chloroform. Liquids that are approximately as viscous as honey can be treated as solids.

3.1.2 Acidic, basic, neutral, and dry extractions, in any combination, can be employed in order to separate diluents or other interferences. These extractions can be performed with commercial products (Toxi-Lab tubes, or equivalent) or laboratory generated solutions.

3.2.0 Liquids. Estimated volumes of liquids need to be in the notes.

3.2.1 For liquid samples with a volume greater than approximately 500ul (15-17 drops). Perform testing using two sampling events, if a GC/MS run is negative the second sampling event does not need to be analyzed.

3.2.1.1 Determine if the sample is aqueous, if the sample is not go to 3.2.4. This may be done through (immiscibility or flame) testing (preferred) or from information submitted with the sample.

3.2.1.2 Determine pH.

3.2.1.3 Use a basic extraction using approximately 0.5N sodium carbonate or 0.5N sodium bicarbonate and an immiscible solvent, chloroform preferred.

3.2.1.4 Samples submitted as controls will be treated the same as the samples they were submitted with.

3.2.1.5 Alternatively, if the sample is neutral an aliquot may be evaporated with air/nitrogen and reconstituted with methanol and/or a methanol-

chloroform mixture.

3.2.1.6 Refer to general drug AM 2.8.0 for reporting conclusions.

3.2.2 For liquid samples with a volume greater than approximately 100ul (3 to 4 drops) but less than 500ul.

3.2.2.1 Determine if the sample is aqueous, if the sample is not go to 3.2.4. This may be done through (immiscibility or flame) testing (preferred) or from information submitted with the sample. If the nature of the sample can't be determined treat it as if it was less than 100ul, section 3.2.3

3.2.2.2 Using ½ of the sample perform a basic extraction using approximately 0.5N sodium carbonate or 0.5N sodium bicarbonate and an immiscible solvent, chloroform preferred. Add internal standard and analyze. Save the second half of the sample and the extract for possible retesting.

3.2.2.3 Samples submitted as controls will be treated the same as the samples they were submitted with.

3.2.2.4 The sample may be evaporated with air/nitrogen and reconstituted with methanol and/or methanol-chloroform, add internal standard, then analyze.

3.2.2.5 Refer to general drug AM 2.8.0 for reporting conclusions.

3.2.3 For liquid samples with a volume less than approximately 100ul (3 to 4 drops), evaporate with air/nitrogen and reconstitute with methanol and/or a methanol-chloroform mixture, add internal standard and analyze.

3.2.4 Organic solvents greater than100ul may be injected directly. For samples less than 500ul, split the sample if possible, add internal standard, analyze and return original and tested sample to evidence. If after the first GC/MS analysis the sample needs to be diluted, dilute with methanol and reanalyze.

3.2.5 If a sample is suspected of containing a controlled substance that is covered by a separate method, i.e. GHB, then that method should be used.

3.3.0 Analysis.

3.3.1 Run samples using a general unknown data acquisition method.

3.3.1 If a peak appears, and is not recognized perform a library search.

3.3.2 If a controlled substance is recognized from a library search or other means, then a standard is run if identity is to be confirmed. Library search reports do not need to be retained in the case file.

3.4.0 Conclusions.

3.4.1 Confirmation. The retention time must be within 0.04 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied then confirmation can be reported.

3.4.2 Non-confirmation. If a standard is not available but the library search produces a match then report "Results of testing indicates the presence of a controlled substance, not confirmed". The reason why the substance is not confirmed must be on the report.

3.4.3 If the RT or MS do not match, or there is no peak at all, then report, "No controlled substances detected".

3.4.4 As with all cases it is up to the analyst to decide whether or not to report non-controlled substances.

4.0.0 FTIR Sample Preparation and Analysis Methods

4.1.0 Direct.

Samples may be analyzed directly with the ATR. Samples may also be mixed with KBr,

pressed into a pellet/window and then analyzed.

4.2.0 Extractions

4.2.1 The organic layer from either a basic or acidic extraction may be mixed with ground KBr, evaporated and analyzed.

4.2.2 Samples undergoing a basic extraction may require bubbling with HCl gas and filtering before HCl salt can be isolated and analyzed.

4.3.0 Analysis

4.3.1 Analyze samples per the General Drug AM section10.

4.3.2 Perform a library or literature search of the resulting spectra.

4.4.0 Conclusions

4.4.1 Confirmation.
If the spectra of the standard in the ISP Forensics produced library and sample match in all significant respects the compound may be reported.

4.4.2 Non-confirmation
If a spectra from an ISP Forensics produced library is not available but the library or literature search produces a match the presence of the compound may be reported with a "not confirmed" statement.

4.4.3 If a spectral match to a controlled substance is not made then the sample must be analyzed on the GC/MS.

4.4.4 The analyst may decide whether or not to report non-controlled substances.

5.0.0 History

Revision #	Issue or review date	History	Author or Reviewer
0	9/20/02	Original Issue	D.C. Sincerbeaux
1	3/13/03	Rev sec 3.2	D.C. Sincerbeaux
2	1/12/07	Rev sec 4.2.1, 3.14	D.C. Sincerbeaux
3	7/2/12	Changed 2.2.0, 3.1. 4.3, 3.3.2, 4.1.0 4.3.1, 4.4.3	D.C. Sincerbeaux
4	2/22/13	Changed 3.1.4, 3.1.4.2, 3.3.2	D.C.Sincerbeaux
5	12/1/14	Changed all of section 3, added 2.5.0	D.C. Sincerbeaux
6	12/16/14	Changed 3.0.0, 3.1.0, 3.2.0, 3.2.1, 3.2.1.5, 3.2.2, 3.2.3.1, 3.2.4, 3.2.5, 3.2.6 (added by number change), 3.3.1 renumbered most of 3.0.0.	D.C. Sincerbeaux