Biochemical Diagnostics Detectabuse® and Multi-Prep® Drug Extraction Troubleshooting Guide

General Comments

Please refer to the notes and supplemental information accompanying each extraction procedure before starting your work.

Sample Preparation:

When preparing the sample it is important to make sure that the proper pH has been achieved before extraction. Improper pH often leads to poor recoveries and/or "dirty" extracts. Samples with a lot of suspended matter or precipitates should be spun down to prevent blockage of the extraction column. In general, when adding an internal standard dissolved in an organic solvent to a sample, the solvent volume must not exceed 5% of the buffered or pH adjusted sample volume. Higher solvent concentrations may cause the formation of precipitates and/or extraction losses.

Hardware:

Hardware maintenance is an important part of achieving reliable results. Hardware should be rinsed and disinfected daily for personnel safety and to prevent deterioration of hardware components. When running methods that require vacuum for column drying it is important that the rubber gaskets be inspected to insure that they are not torn or separated from the column mounting plate.

Make sure that all hose connections are tight and that a non-collapsible vacuum hose is used. Poor vacuum often leads to "dirty" samples and/or poor recovery.

Column Conditioning:

All Detectabuse GV-65 columns must be conditioned with methanol to enable the aqueous sample to make good contact with the resin. Methods that require the cation exchange function to be activated on the GV-65 column must be also be treated with sodium bisulfite.
The GV-65C column is already cation exchange activated and only requires methanol activation.
The Multi-Prep GVSA column (strong anion exchanger) is conditioned differently as per the protocol of each extraction method.

Column Performance:

The GV-65, 96 Deep-well Microtiter plates, and GVSA columns are designed to flow freely by gravity. If liquids do not flow freely there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow. Precipitates or sediment poured onto the column can also hinder or block the flow of liquids through the column.

Sample Extraction:

The extraction methods developed by Biochemical Diagnostics are reliable and rugged. Good results however are dependent upon the quality of reagents, selection of proper containers and closures, and good laboratory technique.

Derivatization:

There are many reliable derivatization techniques being used. When problems arise they are almost always related to a bad reagent, improper drying, or derivatization conditions not optimized

Troubleshooting:

The following troubleshooting section provides in depth solutions to problems that might be encountered when using the Multi-Prep or Detectabuse extraction products. The solutions apply only to the extraction methods. Instrument problems are beyond the scope of this guide. *We do suggest however, that NEAT standards (derivatized if called for in the method) be injected onto the instrument to insure that adequate sensitivity and peak shape is achieved before attempting to analyze samples.*

PROBLEM	CAUSE	SOLUTION (S)
Low Sensitivity	1. Poor recovery caused by improper SPE column conditioning.	Make sure proper column conditioning is done. Methanol will evaporate over time; therefore columns should be used within 30 minutes of conditioning. If cation exchange is used with the GV-65 columns, try a fresh solution of sodium bisulfite. Columns conditioned with methanol and sodium bisulfite should be used within 30 minutes of conditioning.
	2. Improper sample pH	Check pH before adding sample to the column. When using the cation exchange method the pH should be between 2 and 3. 1 ml of 1% HCl is appropriate for fresh samples or samples with a pH range between 5-7. Old samples or samples delayed in transportation often show an increase in pH to the basic side. In this case we recommend a higher quantity of 1% HCl to achieve the desired pH or use 1 ml of buffer solution consisting of 5% monobasic potassium phosphate containing 3% HCl.
	3. Improper sample pH after Amphetamines oxidation	Following oxidation adjust the pH to between 2 and 3 using 250-400µL of 10% HCl and 1mL of buffer solution consisting of 5% monobasic potassium phosphate containing 3% HCl.
	4. Reagent problem.	Check that the proper reagent and reagent volume is used at each step. We recommend the use of color coded labels for marking each reagent. If there is a series of washes within the procedure, mark each reagent with “Wash 1”, “Wash 2”, 3, etc. Prepare a fresh “TEA” reagent every few days.
	5. Incomplete column drying	Methods that require column drying prior to elution are adversely affected by aqueous solutions remaining on the column. A strong pull should be felt on the palm of the hand when it is placed on a column during the drying step. If not, check the pump, tubing connections and gasket on the column mounting plate as described in the general instructions.

PROBLEM	CAUSE	SOLUTION (S)
Values are not as expected	1. Control and/or standard values are incorrect.	Substitute new standard curve and/or controls.
	2. Internal standard error	Make sure that sample volume of internal standard is the same as used for controls and standards. Volumes of 50-100µL can be accurately pipetted. We recommend diluting standards with water for better pipetting and addition of food coloring.
	3. Software programming error	Check the program calculations
	4. Control and/or standard values are incorrect.	Substitute new standard curve and/or controls.
	5. Internal standard error	Make sure that sample volume of internal standard is the same as for controls and standards.
	6. Incomplete hydrolysis results in low recovery	Review hydrolysis procedure. Start with fresh, high purity enzyme and/or fresh reagents if acid hydrolysis. Make sure incubation temperatures and duration are correct. Always mix new solutions as they are added to the hydrolysis mix.
	7. Acid droplets left on walls of Screw Cap tubes following acid hydrolysis.	Allow acid hydrolysis tubes to cool after completion followed by mixing before removing the cap. Mixing after Base addition will help give correct final pH.
	8. Software error	Check the program calculations
	9. Solvent problem	Certain solvents such as Ethyl Acetate may contain organic peroxides that can oxidize sensitive compounds such as THC-COOH. Check for peroxides by preparing a 50% aqueous solution of KI. Mix one mL of this solution with approximately 8 mL of the suspected solvent. Development of a yellow (light contamination) or a brown (heavy contamination) color indicates the oxidation of KI to Iodine. If peroxides are present replace the contaminated solvent.
"Dirty" Chromatograms	1. Incomplete column drying. Methods that require column drying prior to elution are adversely affected by aqueous solutions remaining on the column. These solutions may contain substances that interfere with the chromatography and may actually hinder the flow of organic solvent through the column during the elution step.	A strong pull should be felt on the palm of the hand when it is placed on a column during the drying step. If not, check the pump, tubing connections and gasket on the column mounting plate as described in the general instructions.
	2. Reagent problem.	Check that the proper reagent and reagent volume is used at each step. Prepare a fresh "TEA" reagent and store in a in a closed container.

	3. Improper sample pH	Check pH before adding sample to the column.
PROBLEM	**CAUSE**	**SOLUTION (S)**
Poor chromatography	1. The GC/MS capillary column has deteriorated. 2. Try a different capillary column phase composition for improved peak resolution.	First inject a NEAT standard that does not require derivatization such as Cocaine to test the efficiency of the GC/MS system. If this looks ok follow up with a NEAT, derivatized standard to make sure that derivatization is performing properly.
	3. Derivatizing reagent has deteriorated from being stored at the wrong temperature, from exposure to moisture, and/or exposure to light.	Follow the storage instructions on the package insert. Flush with Nitrogen or Argon after each use. Discard bad reagent and start fresh.
	4. The solvent used to dilute the derivatizing reagent has picked up moisture from the air.	Use a fresh lot of dry solvent.
	5. Improper drying of Propionic acid anhydride, HFBA or PFPA	Dry at higher temps (55°C) and if needed, re-dry after addition of 1mL of Ethyl Acetate or Hexane
Derivatized analytes show little or no sensitivity.	1. The auto-sampler syringe washing solvents are hydrolyzing the derivative. 2. Instrument settings must be optimized 3. Poor instrument maintenance	Methanol remaining in the syringe after the syringe wash step will hydrolyze derivatives sensitive to very polar solvents. It is advisable to follow a methanol syringe wash with at least 3 Ethyl Acetate washes to remove all traces of Methanol. Replace injection sleeve and make sure that the autotune is within specs. Run NEAT standards to narrow down if there is an extraction problem or an instrument problem. If NEAT standards are good then it it most likely not an instrument problem.
Low recovery of Amphetamines	1. High temperature drying or excessive drying 2. Not using Tartaric acid or 0.1% Methanolic HCl	Dry the sample at 37 – 40°C. As soon as the sample is dry, take it out of the heating apparatus. No anhydride residue should be left undried.

Please feel free to contact Biochemical Diagnostics to help you solve your extraction problems. We do not want you to spend time making a research project out of troubleshooting. We have the experience and background to solve most problems over the phone. If necessary, we will visit your laboratory and work with you to solve the problem.

Phone: 1-800-223-4835
1-631-595-9200
180 Heartland Blvd, Edgewood, NY 11717

Fax: 1-631-595-9204
E-Mail: Support@biochemicaldiagnostics.com
www.biochemicaldiagnostics.com