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DETECTABUSE® "NO VACUUM" GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF GABAPENTIN (NEURONTIN) IN SERUM OR PLASMA BY GC/MS

Revised: January 2009
(See: Column Conditioning – Revised GV-65C conditioning)

SAMPLE PREPARATION - *(Please see Notes and Supplemental Information before proceeding)*

1. Add 0.5 mL of sample to a disposable borosilicate glass tube.
2. Add an amount of internal standard equivalent to your drug cutoff level.
3. Add 1.0 mL 10% HCl in deionized water.
4. If adjusted sample is turbid or precipitated centrifuge for 3 minutes at 3000 RPM.

Notes: *When adding an internal standard dissolved in an organic solvent to a urine or blood sample, the solvent volume must not exceed 3% of the buffered sample volume. Higher solvent concentrations may produce extraction losses.*

HARDWARE SETUP - *(Please refer to the Detectabuse Hardware Setup Instructions)*

COLUMN CONDITIONING – (***Follow Column Conditioning procedure for <u>EITHER</u> GV-65 or GV-65C columns.)***

Column Conditioning and Activation of Cation Function using <u>GV-65</u> Columns

1. Wash column with 1.0 mL of Methanol. Allow to flow by gravity.
2. Add 1.0 mL of a Sodium Bisulfite solution to each column. Allow to flow by gravity. ***Prepare by dissolving 5 grams of Sodium Bisulfite in 100 mL of a (1:1) mixture of H2O:0.25M Phosphate Buffer, pH 6.0. Prepare monthly (Store Refrigerated).***
3. Wash column with 1.0 mL H2O. Allow to flow by gravity.
4. Proceed to Sample Extraction within 60 min. of column conditioning.

Column Conditioning using <u>GV-65C</u> Columns

Note: *The GV-65C column is manufactured with the cation exchanger and does not require the addition of Sodium Bisulfite.*
1. Wash column with 1.0 mL of Methanol. Allow to flow by gravity.
2. Wash with 1.0 mL of deionized water. Allow to flow by gravity.
3. Proceed to Sample Extraction within 60 min. of column conditioning.

SAMPLE EXTRACTION - *(Please see Notes at end of this section before proceeding)*

1. Pour samples onto preconditioned column. Allow to flow by gravity. Samples will flow through the column at a rate of 1-2 mL/min.
2. Wash column with 3.0 mL of deionized water. Allow the columns to flow by gravity.
3. Wash column with 0.5 mL of Methanol. Allow the columns to flow by gravity.
4. Add 1.0 mL Ethyl Acetate. Allow the columns to flow by gravity.
5. Proceed to Sample Elution.

Note: *If liquids do not elute freely by gravity flow, there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow.*

SAMPLE ELUTION

1. Sample elution is done outside of the vacuum box.
2. Place the column mounting plate on the elution rack loaded with an appropriate number of 12 x 75 mm or 15 x 85 mm borosilicate glass test tubes. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
3. Add 1.5 mL of Methanol with 2% Ammonium Hydroxide* to each column and allow solvent to flow through the columns by gravity into the test tubes.
4. Add 100 uL of a saturated solution of Tartaric Acid in Ethyl Acetate (1 mg/mL) to each tube, followed by gentle mixing.
5. Dry under N2 or argon at less than 65°C.

*** Elution solvent with 2% Ammonium Hydroxide** (2 mL Ammonium Hydroxide is added to 98 mL Methanol. Prepare daily. Close bottle tightly when not in use.

Note*: If a sample does not elute freely by gravity flow, there is probably air trapped within the column bed or frits. In most cases, tapping the column will initiate flow.*

RECONSTITUTION

1. To each dried extract add 100 µL Methanol.
2. Mix the tube contents, flush with nitrogen or argon and cap the tube or transfer contents into 100 µL reaction vials and seal.
3. Inject 1.0 µL.

SUPPLEMENT - When using an automated robotic system all liquids may be allowed to flow unassisted through the column or may be pulled through the column with vacuum or pushed through with positive pressure.

Assisted flow parameters may be set as follows:
Column Conditioning - Pass through column in approximately 20 seconds (± 20%).
Sample, Sample Washes, and Elution Solvent - Pass through column in approximately 60 seconds (± 20%).

GC/MS ANALYSIS
GC/MS: Hewlett-Packard equipped with Mass Selective Detector
GC Column: H.P. Ultra 2 Capillary Column (or equivalent), 15 m x 0.25 mm, 0.25 µm film thickness
Acquisition Mode: SIM
Temperature Program:
Injector Temp.: 265°C
Detector Temp.: 290°C
Initial: 80°C, Hold for 1.0 min., program at 30°C/min. to 280°C
Equil. Time: 1.0 min.
Split Ratio: Splitless
He Flow: 1.0 mL/min. @ 200°C
Septum Purge: 2 mL/min.
Purge Off Time: 1.5 min.
Dwell: 20
Solvent Delay: 2.5 min.
Start Acq.: 2.5 min.
Stop Run: 7.8 min.

MSD SIM PROGRAM

Drug	Ions Monitored	Retention Time (min.)
Gabapentin	81,110,152,153	4.88

Retention time and ion spectra will vary somewhat from instrument to instrument.

This method is a preliminary procedure for investigational use only. Although it has performed well in our laboratory the method must be validated by your laboratory before it is used to report patient values. We would appreciate your comments on its performance and welcome your suggestions for improvements or enhancements.