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# DETECTABUSE® "NO VACUUM" GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF NICOTINE AND COTININE IN URINE BY GC/MS

*Please see Notes and Supplemental Information before proceeding*

MAY 2010

## SAMPLE PREPARATION

1. Pipet 1.0 mL of sample .
2. Add appropriate amount of internal standard.
3. Adjust pH to 2 with ≈ 1mL of 1% HCl.

**COLUMN CONDITIONING -** *ALL LIQUIDS FLOW BY GRAVITY*

***(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.
2. Add 1.0 mL of a Sodium Bisulfite solution to each column.
   *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 mL Deionized Water.
4. Proceed to Sample Extraction within 20 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
2. Wash with 1.0 mL of deionized water.
3. Proceed to Sample Extraction within 20 min. of column conditioning.

## SAMPLE EXTRACTION

1. Decant samples onto column.
2. Wash column with 3.0 mL of 0.01% HCl.
3. Wash column with 2.0 mL of Methanol.
4. Wash columns with 1.0 mL Ethyl Acetate.

## 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 16 x 100 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 n-Butyl Chloride:Ethyl Acetate (80:20) with 4% Triethylamine (TEA) to each column.
4. Dry under N2 or argon at 40°C. Over drying may cause losses.

## RECONSTITUTION

1. To each dried extract add 100 µl Ethyl Acetate, vortex mix, then flush with nitrogen or argon.
2. Mix the tube contents, and cap the tube or transfer contents into 100 µl reaction vials and seal.
3. Inject 1.0 – 2.0 µl

## MSD SIM PROGRAM

| Drug | Ions Monitored | Retention Time (min.) |
|---|---|---|
| Cotinine | <u>98</u>, 119, 176 | 5.10 min. |
| Cotinine-d3 | <u>101</u>, 122, 179 | 5.00 min. |
| Nicotine | <u>84</u>, 133, 181 | 3.26 min. |

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

## NOTES:

1. **<u>SAMPLES AND WASHES</u>** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.
2. **<u>INTERNAL STANDARDS-</u>** When preparing the internal standard, the quantity added per mL of sample should approximate the cutoff value of the compound(s) being tested for. The Internal Standard can almost always be prepared in an aqueous matrix. If prepared in an organic solvent the solvent must not exceed 5% of the final prepared sample.
3. **<u>TURBID SAMPLES</u>** may need to be centrifuged
4. **<u>RINSE SOLVENTS</u>** should be delivered to the top part of the column to better remove the aqueous.
5. **<u>ELUTION SOLVENTS</u>** with the TEA should be made fresh daily.
6. **<u>POLAR SOLVENTS</u>** used (e.g. acetonitrile and ethyl acetate) may absorb moisture. Flush bottles with nitrogen, keep stock bottles full or use sodium sulfate to minimize moisture.
7. **<u>AIR TRAPPED</u>** within the column bed or frits may prevent the liquids from eluting freely by gravity flow. Tapping the column mounting plate onto the vacuum box should initiate flow.
8. **<u>IDEAL FRAGMENTS</u>** should be determined by full scans of neat, derivatized standards.
9. **<u>RECOMMENDED CAPILLARY COLUMNS</u>** for adequate partitioning are HP-1 or HP-5 or equivalent.

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