



DETECTABUSE® GRAVITY SERIES GV-65 / GV-65C METHOD FOR THE ANALYSIS OF URINARY BENZODIAZEPINES BY GC/MS

Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION

1. Add 2.0 mL of urine to a 16 x 100 mm disposable borosilicate glass tube.
2. Add appropriate amount of the deuterated standards to each sample.

SAMPLE HYDROLYSIS

1. Add 0.5 mL of 0.2M Acetate Buffer, pH 5.0 to each prepared sample. Verify pH 4.5 - 5.0.
2. Add 5,000 units of Beta-Glucuronidase, Helix Pomatia (or equivalent) per mL of sample.
3. Mix gently and incubate at 55°C for two hours.
4. Add 1.0 mL of 0.25M Phosphate Buffer, pH 10.0. Adjust pH of 10 with 2N NaOH or KOH.
5. Centrifuge for 5 minutes at 3000 RPM.

COLUMN CONDITIONING – ALL LIQUIDS FLOW BY GRAVITY

(Follow Column Conditioning procedure for EITHER GV-65 or GV-65C columns.)

Column Conditioning and Activation of Cation Function using GV-65 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.

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Column Conditioning using GV-65C 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. Pour samples onto preconditioned column.
2. Add 2 mL of Water: Methanol (80:20).
3. Dry the columns by applying vacuum adjusted to at least 7" Hg for 10 minutes

SAMPLE ELUTION

1. Sample elution is done outside of the vacuum box.
2. Place the column mounting plate on the elution rack loaded with corresponding labeled number of 12 x 75 mm or 15 x 85 mm.
3. Add 1.5 mL of basic Elution Solvent (Ethyl Acetate:Isopropanol, 95:5) with 2% Triethylamine (TEA)*.
- 4 Dry under N₂ or argon at $\leq 55^{\circ}\text{C}$.

DERIVATIZATION

1. To each dried extract add 50 μL Ethyl Acetate and 50 μL MTBSTFA with 1% TBDMCS.
2. Incubate the mixture @ 70°C for 30 min.
3. Cool, cap and vial.

**MSD PROGRAM
Drug**

**Ions
Monitored**

Desalkylflurazepam	<u>345</u> , 346, 347
Nordiazepam	<u>327</u> , 328, 329
Temazepam	<u>343</u> , 344, 345,372
2- hydroxyethylflurazepam ,	288, <u>290</u> , 389
7-Aminoflunitrazepam	354, <u>355</u> , 356,327
Desmethylflunitrazepam	310, <u>356</u> , 357
7-Aminonitrazepam	<u>380</u> , 381, 382
Oxazepam	<u>457</u> , 458, 459, 513
7-Aminoclonazepam	<u>414</u> , 415, 416, 471
Lorazepam	<u>491</u> , 492, 493, 513
a-Hydroxyalprazolam	<u>381</u> , 382 ,383,396
a-Hydroxytriazolam	<u>415</u> , 416, 417

NOTES:

1. **SAMPLES AND WASHES** – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.
2. **INTERNAL STANDARDS** – 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. **RINSE SOLVENTS** should be delivered to the top part of the column to better remove the aqueous.
4. **ELUTION SOLVENTS** with the TEA should be made fresh daily.
5. **POLAR SOLVENTS** 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.
6. **AIR TRAPPED** 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.
7. **IDEAL FRAGMENTS** should be determined by full scans of neat, derivatized standards.
8. **RECOMMENDED CAPILLARY COLUMNS** for improved partitioning of benzodiazepines would be the newer proprietary drug columns.

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.