Please see Notes and Supplemental Information before proceeding

SAMPLE PREPARATION -

1. Pipet 2.0 mL of sample into a 16 x 100 mm disposable borosilicate glass tube with an inert screw cap.
2. Add 25 ng of PCP-d5 per mL of sample as internal standard.
3. Add 2.0 mL 1% HCl in deionized water. Vortex mix. Adjust pH to 2.
4. If adjusted sample is turbid or precipitated centrifuge for 3 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. Allow to flow by gravity.
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. Proceed to Sample Extraction within 60 min. of column conditioning.

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. 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

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 0.1% HCL water. Allow to flow by gravity.
3. Wash column with 2.0 mL of Methanol. Allow to flow by gravity.
4. Wash column with 1.0 mL Ethyl Acetate. Allow to flow by gravity. Proceed to Sample Elution.

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 n-Butyl Chloride:Ethyl Acetate, (80:20) with 4% Triethylamine (TEA) to each column and allow solvent to flow through the columns by gravity into the test tubes.
4. Dry under N2 or argon at less than 50°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 2.0 µL.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Ions Monitored</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phencyclidine</td>
<td>200, 242, 243</td>
<td>5.50</td>
</tr>
<tr>
<td>Phencyclidine-d5</td>
<td>205, 247, 248</td>
<td>5.48</td>
</tr>
</tbody>
</table>

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

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. **TURBID SAMPLES** may need to be centrifuged.

4. **RINSE SOLVENTS** should be delivered to the top part of the column to better remove the aqueous.

5. **ELUTION SOLVENTS** with the TEA should be made fresh daily.

6. **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.

7. **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.

8. **IDEAL FRAGMENTS** should be determined by full scans of neat, derivatized standards.

*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.*