INSTRUCTIONS

MethElute™ Reagent

TS-49300 TS-49301

Introduction
MethElute™ reagent is a 0.2 molar trimethylanilinium hydroxide in methanol solution. When heated with drug-containing extracts of serum or urine, those drugs containing reactive amino, hydroxyl and carboxy functions will be methylated at these reactive sites. These methylated drugs are then analyzed by gas chromatography.

Product Description

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<th>NUMBER</th>
<th>DESCRIPTION</th>
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<tr>
<td>TS-49300</td>
<td>MethElute™ Reagent, 10 ml Hypo-Vial™ Sample Storage Vial.</td>
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<tr>
<td>TS-49301</td>
<td>MethElute™ Reagent, 12 x 1 ml Hypo-Vial™ Sample Storage Vials</td>
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</table>

These products are packaged under nitrogen. Store at room temperature and free of moisture.

CAS # for methanol 67-56-1
CAS # for trimethylanilinium hydroxide 1899-02-1

NOTE: MethElute™ reagent has been abbreviated with the acronyms TMAH or TMPAH, depending on the system for naming this compound. Some refer to MethElute™ reagent as trimethylphenylammonium hydroxide (TMPAH) to distinguish it by acronym from trimethylammonium hydroxide (TMAH), another commonly used, on-column methylating agent.

Instructions For Use
Normal serum or urine is collected with the normal laboratory precautions.

Materials Supplied
MethElute™ Reagent - 0.2 M trimethylanilinium hydroxide in methanol.

Materials Required
Reagents and Standards for Drug Screen
a. MethElute™ Reagent.
b. 1.0 M sodium acetate buffer, pH 4.80 ± .03.
c. C.P. grade chloroform.
d. Internal Standards.

Obtain pure forms of the drugs (prescription preparations often contain various amounts of fillers).
1. 4 mg methyl stearate per liter of chloroform.
2. 4 mg of methyl laurate per liter of chloroform (CHCl3).
3. Stock solution of desired drugs, 100 mg/100 ml distilled water.
4. Stock Sedulon solution, 1 mg/ml in distilled water.
e. Gas chromatograph equipped with a flame ionization detector (FID). Most commercially available instruments can be used.
   1. Injector temperature: 300°C
   2. Detector temperature: 300°C
   3. Carrier Gas: 30 ml/min helium

f. Columns
   The columns used in these analyses were 3% OV-17 on Gas-Chrom Q 100/120 mesh and 3% SE-30 on Gas-Chrom Q 100/120 mesh. The columns were 6 ft. by 0.08 in. I.D. glass. The columns were conditioned by holding them at an initial temperature of 100°C for 1 hour and increasing the temperature at a rate of 1°/minute until a final temperature of 190°C was reached. The columns were then held at this temperature for 18 hours. The detector end of the column was not connected during this treatment.

g. 15 x 190 mm ground-glass stoppered test tube.

h. Centrifuge and two 12 ml conical centrifuge tubes.

i. Incubator or heating block capable of maintaining 60°C ± 2°C.
   We recommend our dry block Reacti-Therm™ Heating or Heating/Stirring units for heating and incubating samples at a controlled temperature in an environment free of moisture.

j. Syringe capable of accurately injecting 4 µl into gas chromatograph.

Procedure

The following procedure describes the use of MethElute™ Reagent for the derivatization of drugs. Good laboratory practice requires that you establish the separation of drugs on your GC equipment.

Prepare a working drug standard by diluting the stock drug standard containing all the screening drugs 1 to 100 (v/v) with distilled water. The final concentration of each drug is then 1 mg/100 ml. The internal standard is prepared by diluting the stock Sedulon solution (1 mg/ml) 1 to 100 with acetate buffer. These standards should be made fresh each day.

1. To 1 ml of serum, urine or standard in a 15 x 90 mm ground-glass stoppered test tube, add 1 ml of acetate buffer containing 1 mg/100 ml of Sedulon and 10 ml of chloroform. Extract the drugs by shaking the test tube vigorously for 1 minute.

2. Centrifuge the test tubes and remove the upper aqueous layer by aspiration, then discard. Duplicate 4 ml aliquots of the chloroform layer are pipetted into each of two 12 ml conical centrifuge tubes, one labeled "MethElute™ Reagent," the second "CHCl₃". Pipette 1 ml of chloroform containing methyl laurate into the tube marked MethElute™ Reagent, and one ml of chloroform containing methyl stearate into the tube marked CHCl₃. The chloroform is evaporated by placing both centrifuge tubes in a water bath at 60°C and directing a stream of nitrogen over the chloroform.

3. Dissolve the contents of the MethElute™ Reagent centrifuge tube in 25 µl of 0.2 M MethElute™ Reagent. Inject 4 µl of this onto an OV-17 column at 165°C. All of the drugs except meprobamate are detected by this injection.

4. Meprobamate is detected by dissolving the contents of the tube marked CHCl₃ in 25 µl of CHCl₃ and injecting 4 µl onto the OV-17 column at 165°C.

Confirming tests are described below for each of the drugs appearing in the survey runs. The chromatographic conditions are the same for the screening analysis except that the column temperatures are as indicated.

For barbiturates and Doriden, inject 4 µl of the CHCl₃ centrifuge tube on OV-17 at 190°C. Hexobarbital cannot be distinguished from Doriden in the screening tests.

For meprobamate, inject 4 µl of the CHCl₃ tube contents on SE-30 at 155°C.

For Noludar, inject 4 µl of the CHCl₃ centrifuge tube contents on SE-30 at 150°C.

Notes and Precautions

1. Occasionally, a large peak will appear between seconal and hexobarbital in normal serum. The identity of this peak is not known, but generally it does not interfere with the analysis. In any case, no artifacts of any significance have been found in the confirming tests.
2. The screening analysis is useful in cases of suspected drug overdose. The screen includes most of the drugs usually involved in these incidents. If the patient is known to have taken one of the drugs included in the screening test, this test may be by-passed and the drug analyzed directly by the appropriate confirming test.

3. No two columns are packed in precisely the same manner; therefore, temperatures indicated for the various analyses may require adjustment to give optimal resolution.

4. This screening procedure is specifically designed to detect drug overdoses when blood levels of the drugs range from 1 mg/ml for Doriden to 5-10 mg/100 ml for meprobamate and phenobarbital. Greater purification of the serum sample is required to determine therapeutic drug levels below 1 mg/100 ml. For example, in the case of barbiturates: the first chloroform extract should be re-extracted into dilute NaOH, the NaOH acidified and the barbiturates extracted into chloroform, the chloroform dried and the extract injected. Sedulon, Noludar and Doriden will remain in the first chloroform extract; therefore, Sedulon cannot be used as an internal standard.

5. Non-derivatized Noludar has approximately the same retention time as the methyl derivative of secobarbital in the screening analysis. Therefore, the analyst must be certain to verify secobarbital by the proper confirming analysis.

6. 2-dimethyl-xanthines, theophylline and theobromine, after methylation, give caffeine.

7. Good laboratory practice requires that you establish the separation of drugs on your GC equipment.

Calculations

The gas chromatographic response may be measured by a variety of methods; however, because the peaks are symmetrical, it is valid to equate peak height with response.

The coefficient of variation for the procedure is 5% or less with a sensitivity for barbiturates of 0.2 mg/dl or better.

References


Chromosorb is a trademark of Johns-Manville Corp.; Gas Chrom Q is a trademark of Applied Sciences; Dillantin is a trademark of Parke Davis & Co.; Doriden is a trademark of USU Pharmaceutical; Noludar and Sedulon are trademarks of Hoffman-La Roche.

Current versions of product instructions are available at www.thermo.com/columns.

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