INSTRUCTIONS

FLOROX™ Reagent

49650

Number Description
49650 FLOROX™ Reagent, 10 ml, contains 2.5 mg/ml of 0-(pentafluorobenzyl) hydroxylamine•HCl in EC grade pyridine

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

FLOROX™ Reagent couples a highly EC sensitive electrophore directly to keto functions that results in low level (0.1 ng) determinations of ketosteroids.

Example Procedures

Protocol I: Flame ionization detection of 1-10 µg of steroid
1. Add 0.1 ml FLOROX™ Reagent to 1-10 µg ketosteroids isolated as a residue in a 1.0 ml Reacti-Vial™ Small Reaction Vial.
2. Cap vial and heat at 65°C for 1 hour; allow reaction to cool.
3. Evaporate reaction mixture with nitrogen.
4. Add 0.1 ml cyclohexane to the vial, then 0.1 ml water.
5. Cap vial and shake.
6. Remove upper cyclohexane layer for FID-GC.

Protocol II: Electron capture detection of 12.5-50 ng of steroid
1. Add 10 µl FLOROX™ Reagent to 12.5-50 ng steroid residue in a 1 ml Reacti-Vial™ Small Reaction Vial.
2. Cap vial and heat at 65°C for 1 hour; allow reaction to cool.
3. Evaporate reaction mixture with nitrogen to remove pyridine.
4. Dissolve residue in 0.5 ml cyclohexane.
5. Wash with 0.5 ml cyclohexane.
6. Transfer cyclohexane layer to another vial; dry reaction mixture by adding anhydrous Na₂SO₄.
7. Use cyclohexane phase for EC-GC.
Protocol III: Electron capture detection of 0.1-5 ng of steroid

1. Add 10 µl EC grade pyridine and 2 µl FLOROX™ Reagent to 0.1-5 ng steroid residue in a 1 ml glass-stoppered tube.
2. Cap tube and heat at 65°C for 1 hour; allow reaction to cool.
3. Evaporate reaction mixture with nitrogen to remove pyridine.
4. Dissolve residue in 25-100 µl EC grade cyclohexane.
5. Wash with an equal volume of 10% HClO₄.
6. Centrifuge tube and remove sample from upper layer for EC-GC.

   Note: Short columns with low loadings are used for the analysis (1% OV-17 on GAS CHROM Q 100/120 mesh, 18” long by 3 mm I.D.). If desired, non-derivatized hydroxyl groups may be silylated after the pyridine removal step.

References
