Waters
Lab Highlights
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A SEP-PAK® CARTRIDGE CLEANUP OF URINE FOR ANALYSIS OF MARIHUANA METABOLITES

The widespread use of marihuana has facilitated the investigation into rapid, quantitative and reliable methods for its detection in urine for routine forensic use. Marihuana's principal active component, Δ^9-tetrahydrocannabinol rapidly disappears from the blood and is metabolized to mostly 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (9-THC-COOH) and excreted in the urine. The use of GC, HPLC or TLC as any one analysis method is not sufficient for legal purposes, and only GC/MS is acceptable for 9-THC-COOH detection, but only after sample cleanup. SEP-PAK® C_{18} cartridges have recently been used (1) as a sample prep method for 9-THC-COOH in urine. Sample prep of the urine for analysis has been previously done by TLC or liquid-liquid extraction. The method uses a methylation reaction in order to derivatize the 9-THC-COOH and consists of the steps shown in Part I, II, and III.

Recovery of sample was investigated using 8-THC-COOH with mean recovery of 9.8% ± 3.3% S.D. for 200 ng, based on a replicate of five samples. Background interferences in the MS are greatly reduced as evidenced by the detection limit of 10 ng/ml of 9-THC-COOH.

The authors concluded by saying, "The advent of these disposable cartridges presages a wider use in analytical toxicology because of a number of inherent advantages. They provide a rapid extraction of a relatively large volume of urine with a small volume of solvent. The recovery of a polar cannabinoid is virtually quantitative and the results show excellent cleanup and reproducibility. We feel that further adaptions of these cartridges to the extraction of other drugs-of-abuse and organic poisons in biological materials are in the offing."

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Part I - Alkaline Hydrolysis

1. A 10 ml of urine is hydrolyzed by adding 1 ml of methanolic KOH (10% w/v) to a flask.

2. The flask is capped with aluminum foil, placed in an oven for 15-20 min. @ 100°C.

3. Cool flask in a cold water bath and add 1.5 ml of glacial acetic acid to adjust pH to 3.0-4.0.

Part II - Extraction

1. Wet SEP-PAK® cartridge with 2 ml CH_3OH, then 2 ml H_2O. Hydrolysate from Part I is passed through cartridge at a rate of 5 ml/min.

2. Wash cartridge with 5 ml H_2O, then 5 ml of ACN:H_2O (40:60 v/v), then 2 ml of CH_3OH to elute 9-THC-COOH and evaporate to dryness under N_2 on hot water bath.

Part III - Esterification

1. The dried extract from Part II was treated with 70 µl of 25% tetramethylammonium hydroxide-dimethylsulfoxide (1:20) (2). Agitate mixture on a vortex, mix and allow to stand for 2 minutes.

2. Add 5 µl of iodomethane, agitate, then allow to stand for 5 minutes. Acidify with 0.2 ml of 1.0M acetic acid.

3. Add 1 ml of cyclohexane and agitate for 1 min. Centrifuge if necessary. Remove and save upper layer, re-extract lower layer with 1 ml of cyclohexane. Combine extracts and evaporate to dryness under N_2. Redissolve in 20 µl of methanol before injection.

**FOR INVESTIGATIONAL USE ONLY.
THE PERFORMANCE CHARACTERISTIC FOR THIS PROCEDURE HAS NOT BEEN ESTABLISHED.**

1. Nakamura, G. R., Stall, W. J., Masters, R. G., and Folen, V. A. , *Anal. Chem.* 57 (1985) 1492-1494.
2. Whiting, J. D., Manders, W. W., *Aerosp. Med.* 54 (l983) 1031.