

PART #: WSH96XCE11 - 100 MG 96 WELL PLATE
 PART #: WSH48XCE11 - 100 MG 48 WELL PLATE



1. SAMPLE PREPARATION

To 1-2 mL urine add 500 μ L of pH=6.0 + 0.5 0.1M Phosphate buffer.

Add appropriate volume and concentration internal standards.

Note 1: See alternate hydrolysis step for opiate samples).

(Alternate Hydrolysis Step)

To 1-2 mL urine sample add 500 μ L of acetate buffer (pH= 5.0) containing 5,000 units/mL -glucuronidase. **Optionally**, add 500 μ L of acetate buffer and 25 μ L of concentrated -glucuronidase.

Vortex and heat for 1-2 hours at 65 $^{\circ}$ C.

(Hydroxylamine can be added to sample if oxime derivative is preferred.)

Allow sample to cool.

Do not adjust pH- sample is ready to be added to extraction column.

2. APPLYING SAMPLE TO COLUMN

Load sample directly to column without any preconditioning.

Pull sample through at a rate of 1-2 mL/ minute.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure

(~ 80-100 psi) for 1 minute.

3. WASH

Wash sample with 1 mL of 2% acetic acid/ 98% methanol.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure

(~ 80-100 psi) for a minimum of 5 minutes.



EXTRACTION OF BASIC DRUGS AND METABOLITES FROM URINE USING SPE WELL PLATES



NOTE 2: (It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)

4. ELUTION

Elute samples with 1 mL Methylene Chloride/ Iso-propanol/ Ammonium Hydroxide (78/20/2).

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 30-40 °C.

GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 30 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

Representative Analytes Extracted

Amph/Methamph	MDMA/MDA/MDEA	Opiates (7)	Methadone/EDDP
Sympathomimetics	Meperidine/Normeperidine	Phencyclidine	Cocaine/Benzoylecgonine
Tricyclic Antidepressants (7)	Cyclobenzaprine	Fentanyl/ Norfent	Sertraline
Tramadol/Nortramadol	Diphenhydramine	Citalopram	Clonidine