Steroid Extraction Protocol – Liquid Samples

INTRODUCTION

This protocol serves as a general guideline for extracting steroids from serum, plasma, saliva, and other liquid samples, as well as determining extraction efficiency. Specific steps may require adjustment depending on your sample and experimental setup. Always validate your extraction procedure before applying it to high-value samples.

MATERIALS AND EQUIPMENT NEEDED

- DetectX® kit-specific Steroid standard for extraction efficiency determination
- DetectX® kit-specific 1X Assay Buffer
- ODS/C18 Cartridges (i.e., Bond-Elut™, Sep-Pak™, etc.)
- Positive pressure apparatus for ODS/C18 cartridges
- Methanol (≥ 95% purity)
- Deionized water
- Test tubes capable of holding diluted sample volume
- Container capable of holding cartridge effluent volume
- Optional: Centrifugal vacuum device

PROTOCOL

An ODS/C18 cartridge is required for each sample. Activating, loading, washing, and eluting the ODS/C18 cartridges should be done under slight positive pressure to obtain a flow rate of 0.5 mL/minute. Positive pressure can be achieved through different methods, such as a manifold system, syringe plunger, etc.

1. Make a suitable volume of 20% methanol in water (%V/V). The required volume is dependent upon the number of samples being processed. 6 mL of 20% methanol is needed for each sample.
2. In a clean test tube, dilute samples 1:1 by adding equivalent volumes of deionized water and sample. Vortex for 10 seconds.
3. Activate and equilibrate ODS/C18 reverse phase cartridges by sequentially adding 5 mL of 100% methanol followed by 5 mL of deionized water. Collect the effluent in a waste container.
4. Load diluted samples onto the activated cartridges.
5. Wash each cartridge by loading 3 mL of 20% methanol onto the cartridge, collecting the effluent in a suitable container. Repeat this step for a total of 2 washes. Before eluting samples, ensure cartridges are completely dry by drying under positive pressure for 2 - 5 minutes.
6. Elute samples from the cartridges by adding 3 mL of 100% methanol. Collect the eluate in a clean tube.
7. **If analysis is to be carried out immediately**
    a. Evaporate eluted samples from step 6 completely using a centrifugal vacuum device.
    b. Reconstitute samples with 1X Assay Buffer. To maintain the original concentration of the sample, the reconstitution volume should equal the starting sample volume prior to dilution in step 2.
    c. Briefly vortex and incubate the sample for 5 minutes at room temperature.
    d. Repeat vortex and incubation 3 times to ensure the steroid has solubilized completely.
    e. Continue to dilute samples using 1X Assay Buffer as needed (Assay Dilution Factor).
    f. Proceed directly to Step 9.
8. **If analysis is to be delayed**
    a. Store eluate at -80°C. When ready to process the samples, proceed to step 7a.
9. Run samples in the assay according to the assay protocol (Assay Concentration).
10. Calculate analyte concentration of each sample using the equation below (see Extraction Efficiency section for details on this calculation). Concentration units are assay dependent.

$$\textbf{Analyte Concentration}\left(\frac{\textbf{pg}}{\textbf{mL}}\right) = \frac{\text{Assay Concentration}\left(\frac{\text{pg}}{\text{mL}}\right) \times \text{Assay Dilution Factor}}{\text{Extraction Efficiency}}$$

EXTRACTION EFFICIENCY

To obtain a more accurate reading of the analyte concentration of the sample, the extraction efficiency must be calculated. To determine extraction efficiency, one sample will undergo the extraction process twice; once with a known amount of analyte added (Spiked Control) and once with an equivalent volume of 1X Assay Buffer added (Unspiked Control). A third control (Assay Buffer Control) must also be prepared but does not undergo the extraction process. All aliquots must have equivalent volumes (V1) and all spikes must have equivalent volumes (V2). The preparation of these controls is summarized in the table and figure below.

The three controls are then run in the assay according to the assay protocol. Extraction efficiency is calculated using the following formula:

$$\text{Extraction Efficiency (\%)} = \frac{(\text{Spike Control Concentration} - \text{Unspiked Control Concentration})}{\text{Assay Buffer Control Concentration}} \times 100$$

Extraction Condition	Spiked Control	Unspiked Control	Assay Buffer Control*
Aliquot (V1)	Sample	Sample	1X Assay Buffer
Spike (V2)	Steroid Standard	1X Assay Buffer	Steroid Standard

*The Assay Buffer Control does not undergo the extraction process. Prepare this control immediately prior to assaying samples.

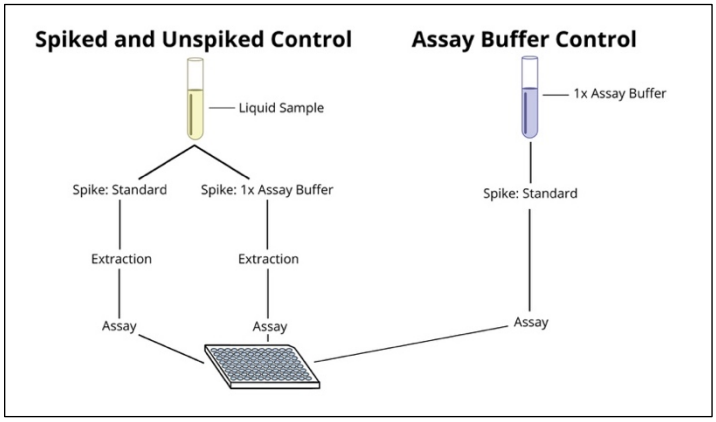

Figure 1. Extraction efficiency workflow diagram for liquid samples.