Team: 16
Version: 1
Date: 27/04/2023

Standard Operating Procedure

Protocol Title: Ninhydrin Test for Detection of Primary and Secondary Amines such as presence of Ketamine(or benedryl) in solutions.

Brief Description of Protocol: The protocol amis to conduct the Ninhydrin test to determine the presence of primary and secondary amines in a sample solution that contains drugs such as ketamine or benedryl. 5 samples ranging from 1mg to 5mg each in a solvent(ethanol or acetone or water). Each sample solution will be stored in an individual test tube. Then prepare the ninhydrin powder and dissolve in the solvent to prepare the Ninhydrin reagent. Heating and cooling methods are used to test the samples and interpretation of the results is done by observing the color change. Purple bluish color means that Amines are present in the solution (i.e ketamine or benedryl is detected). If no color change it indicates the absence of amines. Further the protocol also amins to detect the minm detection level of Ketamine or Bendryl by analysing the color change in the sample solutions. This wil provide deep insight into the experiment such as the sensitivity of the test for detection of drugs such as Ketamine or Benadryl.

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I. **Protocol Title:** Ninhydrin Test for Detection of Primary and Secondary Amines such as presence of Ketamine(or benedryl) in solutions

II. **Materials:**

- **Equipment**
  - Glass vials
  - 100mL glass bottle
  - Test tubes
  - Di water
  - Weighing boats
  - Beakers
  - Pipettes
  - Measuring scale
  - Heating block or water bath oven (set at 110-120℃)

- Reagents and Chemical:
  - Ninhydrin Solution
  - Sample Solution
    - Ketamine sample (Controlled substance and cannot be easily accesed)
      - Benedryl sample (An easy substitute available in market)

III. **Procedure:**

A. Sample Preparation

1. Weigh the sample 2-5 milligrams and transfer it to a container
   a. Do the test for 1mg, 2mg, 3mg, 4mg, 5mg to check for minimum detention level.
2. Transfer each sample to a separate containers (Total of 5 samples)
3. Add and dissolve a small amount of ethanol or other suitable solvents (i.e. acetone or water) to a concentration of 0.1-1.0 mg/mL and mix well to dissolve the samples.
4. Now Transfer the sample solution to a clean test tube.

B. Preparation of Ninhydrin Reagent

5. Weigh out 0.2g of Ninhydrin powder and transfer it to a 100 mL glass bottle.
6. Add 25mL of solvent (ethanol, acetone, or water) to the bottle.
7. Close and cap the bottle and shake vigorously until the Ninhydrin is completely dissolved.
8. Shelf life: The solution can be stored in a tightly sealed container at room temperature for up to 6 months.

C. Testing the Sample

9. Place the test tube containing the ketamine (or benedryl) solution in a heating block or oven set at 110-120℃
10. Add 1-2 drops of Ninhydrin reagent to the sample solution using a dropper or pipette.
11. Remove the test tube from the heating block or over and allow it to cool to room temperature.

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12. Observe the sample solution for a color change. A purple/violet bluish color indicates the presence of amino acids which indicates the presence of ketamine (or benadryl).

D. Interpretation of Results

13. If the sample turns purple/violet bluish, it indicates the presence of amino acids.
14. If the sample remains colorless, it indicates the absence of amino acids which also means that ketamine (or benadryl) is absent.

E. Data Analysis

15. After obtaining the results plot a graph where x-axis is the (1-5mL) and y-axis is the color intensity. Other possible parameters on the y-axis can be absorbance and percentage of detection.
16. Color intensity: To measure the intensity of color change in the sample solutions after adding Nindhyrin. Use a colorometric measurement or optical density by assigning the numerical value to represent the intensity. This allows to see if the intensity of color change varies with different sample sizes.
17. Absorbance: Use a spectrophotometer to measure the absorbance of the sample solution at different or specific wavelengths. This allows to quantify the amount of reaction product formed because of presence of amines. Relationship between two variables (i.e absorbance vs sample size)
18. Percentage of Change or Detection: Assign a 100% to a sample which shows purple bluish color and 0% to the baseline sample where no color change is observed. Positive detection will be assigned a percentage according to the color intensity. Relation between two variables (i.e. percentage of detection vs sample size)

IV. Documentation:

1. Record all the 5 samples in a laboratory notebook and make a electronic version
2. Restore and retain any remaining sample to be used in future experiments or even for reference if needed.

V. References:

- Journal of Chemical Education
- *Ninhydrin Test*. Available at: https://www.toppr.com/guides/chemistry/ninhydrin-test/#:~:text=The%20ninhydrin%20test%20is%20a,presence%20of%20an%20amino%20group. (Accessed: 27 April 20232)
- Sapkota, A., Ouma, E. and Liam (2022) *Ninhydrin test- definition, principle, procedure, result, uses*, *Microbe Notes*. Available at: https://microbenotes.com/ninhydrin-test/ (Accessed: May 2023). .