Aim: Estimation of sulphanilamide by colorimetry

References

1. Indian Pharmacopoeia, 2022, Vol. I & II.
2. Vogel's Textbook of Quantitative Chemical Analysis, 6th Edition.
3. Beckett & Stenlake. Practical Pharmaceutical Chemistry, 4th Edition.

Principle:

Sulphanilamide, a sulfonamide antibacterial drug, forms a colored complex with diazotized reagents, which can be quantified by measuring absorbance at a specific wavelength using a colorimeter or UV-Vis spectrophotometer. The intensity of the color is proportional to the concentration of sulphanilamide in the sample.

Reagents required:

1. Sulphanilamide standard solution
2. Sodium nitrite solution (0.1% w/v)
3. Hydrochloric acid (1N)
4. Ammonium sulfamate solution (0.5% w/v)
5. N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) solution (0.1% w/v)
6. Distilled water

Apparatus required:

1. Colorimeter or UV-Vis spectrophotometer
2. Glass cuvettes
3. Pipettes and burettes
4. Volumetric flasks (10 mL, 50 mL, 100 mL)
5. Test tubes
6. Beakers

PROCEDURE:

1. Preparation of Standard Sulphanilamide Solution:

- Weigh accurately 100 mg of sulphanilamide and dissolve it in 100 mL of distilled water to prepare a stock solution of 1000 µg/mL.
- Pipette 10 mL of this stock solution and dilute it to 100 mL with distilled water to obtain a working standard solution of 100 µg/mL.

2. Preparation of Calibration Curve:

- Pipette out 1, 2, 3, 4, and 5 mL of the working standard solution into separate 10 mL volumetric flasks.
- Add 1 mL of 1N HCl followed by 1 mL of 0.1% sodium nitrite solution to each flask.
- Allow the solution to stand for 5 minutes for diazotization.
- Add 1 mL of 0.5% ammonium sulfamate solution and let it stand for 2 minutes.
- Add 1 mL of 0.1% NEDA solution and dilute to 10 mL with distilled water.
- Allow the color to develop for 10 minutes.
- Measure the absorbance of each solution at 540 nm using a colorimeter or UV-Vis spectrophotometer against a reagent blank.
- Plot a calibration curve of absorbance vs. concentration of sulphanilamide (µg/mL).

3. Estimation of Sulphanilamide in Sample Solution:

- Prepare the sample solution by dissolving an appropriate amount of the given formulation in distilled water.
- Treat the sample solution using the same steps as for the standard solutions.
- Measure the absorbance at 540 nm.
- Using the calibration curve, determine the concentration of sulphanilamide in the sample.

Calculations:

From the calibration curve, determine the concentration of sulphanilamide in the sample solution:

Where:

$$C = \frac{A-B}{m}$$

- **C** = Concentration of sulphanilamide (µg/mL)
- **A** = Absorbance of the sample solution
- **B** = Intercept of the calibration curve
- **m** = Slope of the calibration curve

Calculate the amount of sulphanilamide in the given formulation accordingly.

Sample result:

The calibration curve was obtained by plotting absorbance vs. concentration of sulphanilamide. The equation of the standard curve was found to be:

Where:

- **y** = Absorbance
- **x** = Concentration of sulphanilamide (µg/mL)

For the given sample solution, the absorbance was found to be 0.277. Using the equation:

Thus, the concentration of sulphanilamide in the given sample was found to be 11 µg/mL.

Precautions:

1. Prepare all reagents freshly to ensure accurate results.
2. Maintain the same reaction time for color development in all samples.
3. Handle all chemicals, especially acids and diazotizing reagents, with care.
4. Use the same cuvette for all absorbance measurements to reduce variability.
5. Ensure proper calibration of the colorimeter or UV-Vis spectrophotometer before measurements.