

# Derivative UV Spectroscopy

Experiment 4 - Practical Notes

Name: \_\_\_\_\_ Roll no.: \_\_\_\_\_ Date: \_\_\_\_\_

## Aim

To determine the assigned drug by validated derivative-UV calibration.

## Principle

A derivative spectrum describes the rate of absorbance change with wavelength. It can improve separation of overlapping spectral features when a validated derivative method is used.

## Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

## Procedure

### 1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

### 2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

### 3. Make the measurement

Apply the prescribed validated derivative-UV calibration method and record assigned derivative amplitude at the stated wavelength. Record replicate readings and any interfering appearance.

### 4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

### 5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

**Pharmaceutical Analysis****Observations and Record**

| Measurement                                            | Reading |
|--------------------------------------------------------|---------|
| Test portion / dilution                                | _____   |
| Blank / standard                                       | _____   |
| assigned derivative amplitude at the stated wavelength | _____   |
| Calculated content and unit                            | _____   |

**Worked Example**

The numbers below are illustrative; enter your own observations in the record.

- For an assigned linear calibration  $D = mC + b$ ,  $C = (D - b)/m$ . Apply all dilution factors.
- Example:  $D = 0.030$ ,  $b = 0.002$  and  $m = 0.004$  per microgram/mL give 7.0 micrograms/mL. Record derivative order, wavelength interval and processing parameters.

**Result**

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

**Precautions**

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

**Viva Questions and Answers****1. What produces the analytical signal?**

A derivative spectrum describes the rate of absorbance change with wavelength. It can improve separation of overlapping spectral features when a validated derivative method is used.

**2. Why include a blank or control?**

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

**3. What must accompany the result?**

Units, method, dilution basis, relevant control performance and any limitation.

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