

Standard Operating Procedure

Title: HPLC - Reproducibility, Column Performance and Testing Guidelines

1.2. Expected value

The Relative Standard Deviation for Peak Areas should be less than 2%. The Relative Standard Deviation for the Retention Times should be less than 1%.

1.3. Detector Checks

The power supply and intensity of the Detector lamp play important roles in determining the intensity of signal output which in turn affects the peak area recorded, and thus the calculated concentration of sample. Consult the various manuals in order to check the recommended outputs for power supply and lamp intensity.

The test methods may have the provision to use Photodiode array detectors (PDA), Fluorescence detectors, Electrochemical detectors (ECD), Refractive index detector (RI), and Evaporative Light Scattering detector (ELSD).

The PDA detector is checked for conformance using an internally designed Empower diagnostics method. To run the diagnostics test, from the Quickset screen, choose the PDA diagnostics option, select all tests and run.

The Waters 470 Scanning Fluorescence detector is controlled by monitoring the powering up displays and the hours lamp is used.

The RI is checked to ensure its temperature is 35°C and has been autozeroed

The ECD is controlled by checking that working electrode has been cleaned and the reference electrode solution changed 6 monthly

The ELSD is checked by running the diagnostic tests for the Optics, Nebuliser and Flow meter.

See section 1.4 Recording results for details on record keeping requirements.

1.4. Recording results

The Reproducibility results and the Detector checks results are to be transferred onto the appropriate Calibration of HPLC form (**Form-720 HPLC Records, page 1 or 2**). Compile a print out of the Empower Calibration summary report for the standard injected and file together with the completed form, in the Calibration records folder for the specific HPLC system. Complete also the HPLC Calibration Log (**Form-720 HPLC Records, page 3**) kept at the beginning of the Calibration records folder.

For the HPLC systems used on a regular basis by the same Laboratory Technicians, it is the responsibility of these Laboratory Technicians to ensure that the form HPLC Calibration Log (**Form-090 HPLC Records, page 3**) is completed on a weekly basis, even if calibration was not done on the system. This would assist with the monthly house keeping audit follow through.

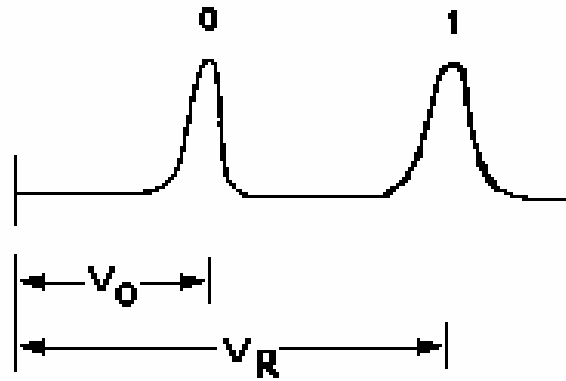
Note:

If the HPLC is found to be out of Calibration follow in order the following steps:

- (a) Record an explanation of problem on the HPLC Calibration Log (**Form-720 HPLC Records, page 3**).
- (b) If the fault can be singled out as user error, or is rectified simply (eg. Air bubble in detector), correct the fault and then re-do the Calibration/ Detector check, fill out a second Calibration of HPLC form (**Form-720 HPLC Records, page 1 or 2**) and attach to first form. Confirm Calibration by adding entry onto HPLC Calibration Log (**Form-720 HPLC Records, page 3**).
- (c) If the fault cannot be singled out, refer concerns to HPLC service person for repair or advice. If the instrument will need repair, place an **"Out of Calibration - Do not use"** label on the HPLC.
- (d) Inform Laboratory Supervisor.
- (e) Ensure the HPLC is repaired. File the completed service report in the Service & Repair folder for that system/s.
- (f) Re-confirm Calibration of the HPLC by repeating section 1 of this SOP.

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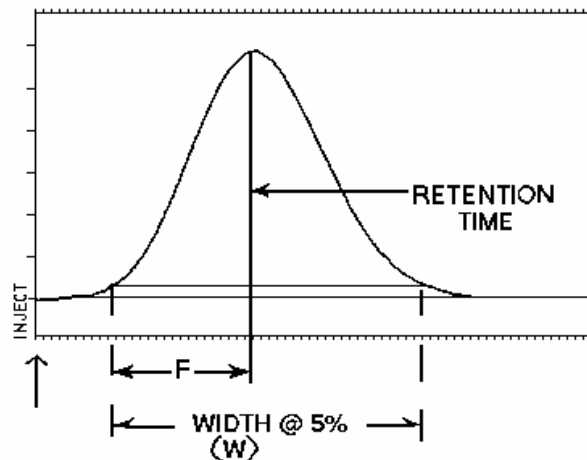
2.4. USP Tailing

The Tailing factor, T is an indicator of peak skewness and is calculated using the equation:

$$T = \frac{W}{2F}$$

Where: W = Peak width at 5% of peak height

F = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height.



2.5. Efficiencies and Column Performance methods for Columns and Cartridges

The Efficiencies in the table below are a guide as to what to expect when a column is new and is tested using the Column performance tests listed below: