if the number of patients is increased to 10, some other values will be added. Say 200, 200, 200, 205, 115.5 are the values to be added. This will change the average to 242.05.

Average

Average is the sum of observations divided by number of observations. Average or mean is the most widely used statistical term. It is denoted by the symbol $\bar{X}$. In the above example the individual values were added and divided by the number of observations to find the average or mean value. Hence the average or $\bar{X} = 242.05$.

Median

Median is the value that divides the data into two halves. Hence median is the 50 percentile of a distribution. Suppose we have a set of 5 numbers 5, 7, 11, 17, 30.

Numerical mean is $(5 + 7 + 11 + 17 + 30)/5 = 14$ but the median will be the number 11, as 2 values 5 and 7 are lower than 11 and 2 values 17 and 30 are higher than 11. Median does not consider the value of the data. It is the midpoint value from which 50% of data are greater while 50% are lesser in value.

In percentile calculation, data are ranked in order of magnitude. The *n*th percentile denotes a value below which *n* percent of data are found. A rank of 90 percentile in an examination means the score is higher than that of 90% students or 90% of the students have scored below that particular student.

Mode

Mode is the value that occurs with the greatest frequency. Let us assume a data set of 9 values; 2, 5, 9, 7, 10, 7, 12, 7, 12. Here, out of 9 values, the value 7 occurs maximum number of times. All other values are less frequent than 7. So, the number 7 is the mode.

Range

In a given data set, range is the difference between the smallest and the largest value. If we have a data set of 1, 3, 5, 7, 9, 11, the range is 1 to 11.

Precision

Precision is a measure of variability; lesser the variability, greater is the precision. For determining an unknown parameter like disintegration time or potency of a tablet, experiments are repeated several times. The outcome obtained from these repeat experiments may vary and the result is expressed as mean of all the results. Let's suppose a tablet is assayed by two methods. Results obtained from the first method are 98, 100, 102 and mean is 100. The results from the second method are 96, 98, and 106 which also shows an average of 100. The extent of variability is more in the second method. Hence, it is less precise though the mean is same, i.e. 100 in both the methods.

Accuracy

Accuracy refers to the closeness of an individual observation to the true value. Let the real potency of a tablet be 100 mg. Two students are given the task of assaying the tablets.

Void time (T_M): Void time is the residence time of a component, which is not retained by column. It is the time of first disturbance in the baseline caused by the sample solvent, which is also called hold up time. In Fig. 10.6, a small peak is observed at 1 minute. This peak indicates the hold up time is 1 minute.

Peak Height and Width (h, w_b)

The concentration of analytes is measured from the peak areas of the chromatograms. The height of the peak represents the highest concentration of the solute. Peak area is roughly equal to $1/2 \times w_b \times h$. The peak width is usually measured at the base (w_b) or peak half height. To measure the w_b, two tangent lines are drawn from the steepest inflection points of the peak. The distance between the two points at which the two tangents cut the baseline is w_b. The width at half height is easier to measure and is used for determination of column efficiency. The height or area of a peak is proportional to the amount of analyte. Since, it is difficult to measure peak width at the base, a relationship with width of half height is used (Fig. 10.7).

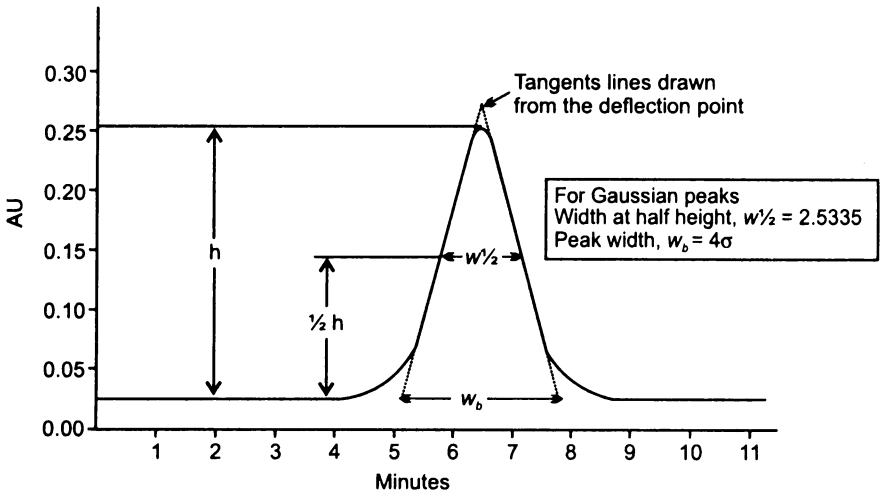

Fig. 10.7: Determination of height and width of a Gaussian peak

Peak volume: Popularly known as bandwidth, it is the volume of the mobile phase containing the peak. Peak volume is the volume of the mobile phase that passes through the detector when the peak is recorded. It is obtained by multiplying the flow rate (F) with peak width (w_b). For an eluting peak, greater the w_b, greater is the peak volume.

$$\text{Peak volume} = w_b \times F \qquad \text{(Eq. 10.1)}$$

So greater the height or lesser the peak volume or sharper will be the peak.

The Retention Volume (V_R)

It is the volume of mobile phase needed to elute an analyte at a given flow rate. The retention volume is found by multiplying the retention factor (T_R) with flow rate (F).

column placed before an analytical column. In pharmaceutical experiments, often samples of biological origin, e.g. blood, tissue extracts, etc. are analyzed. These samples are usually small in volume and difficult to clean up. If they are fed into the main column directly, they may contaminate the packing materials and may be retained into the column. Use of guard column is recommended for such dirty samples. Usually, guard columns are packed with the same materials as the main analytical column. Attaching guard column helps in sample cleaning but they should not cause significant increase in operating pressure. Sometimes, a special type of guard column known as 'scavenger column' is placed between the pump and injector. Here rather than cleaning of sample, the purpose is to protect the analytical column from mobile phase contaminants. Columns that use extremely small particles as packing material get clogged easily. While passing through the scavenger column, mobile phases get cleaned up and chance of clogging is reduced.

Detectors

While the column separates the complex mixture into simple solutions, the detector measures the concentrations of these solutions (eluting analytes). The detector recognizes the analytes by monitoring one of its inherent properties. UV/Vis and photodiode array detectors are among the most commonly used detectors.

UV/Vis detectors: UV/Visual detectors measure the absorption of UV or visible light by HPLC eluents. As most of pharmaceuticals show UV absorption, this detector is widely used in Pharmacy. It usually consists of a deuterium lamp (radiation source), a monochromator (movable gratings or prism that allows only lights of particular wavelength to pass through the exit slit) and a small flow cell through which the mobile phase flows. Normally, dual beam design is used. The light generated by the lamp is divided into two halves, of which only one half flows through the sample cell. Intensity of the both the beams is measured by separate photodiodes. In contrast to normal spectrophotometers, which have bigger flow cells (1–3 ml), flow cells used in HPLC units are small in volume. Typical volume of HPLC flow cells at 2–10 μL and path length is 2–10 μm. Usually, high quality quartz cells are used.

Quantification is done by measuring the absorbance, which is defined as the negative logarithm of transmittance. It is the ratio of the transmitted light to that of incident light. When 90% light is absorbed, absorbance value is 1. The benchmark noise level is typically $1.0 \pm \times 10^{-5}$. The high end of linear absorption range is typically set at 1 to 1.5 but modern and sophisticated instruments have lower noise level and extended high ends (maximum absorbance measurable). The sensitivity of measurement depends on extinction coefficients of the analytes. Higher the extinction, greater is the sensitivity of the method (Table 10.2).

Photodiode Array Detectors (PDA)

In contrast to single wavelength detectors, which record the absorption at single wavelength, PDA detectors provide complete UV spectra of eluting peaks. In short, it performs as a multi-wavelength UV/Vis absorbance detector and is preferred for method development. In UV/Vis detector, the spectrum of deuterium lamp is grated

11 Nuclear Magnetic Resonance Spectroscopy

THEORY OF NUCLEAR MAGNETIC RESONANCE

Nuclear magnetic resonance spectroscopy is the analytical method to find out the number of magnetically distinct atoms in a given compound. All the atoms contain nuclei and all the nuclei contain charge. Some of the charged nuclei possess 'spin'. They are those nuclei which contain either an odd atomic number or odd atomic mass or both, e.g. ${}^{1}_{1}H$, ${}^{2}_{1}H$, ${}^{13}_{6}C$, ${}^{14}_{7}N$, ${}^{17}_{8}O$, ${}^{35}_{17}Cl$, etc. The nuclei which have even number of atomic mass and charge are not suitable for NMR spectroscopy, e.g. ${}^{12}_{6}C$, ${}^{16}_{8}O$, etc. Those nuclei which possess spin, they spin on their nuclear axis which generates a magnetic dipole 'μ'. The angular momentum of this spinning charge can be quantified and described in terms of quantum spin number 'I'. Angular momemtum of a particle about an origin is a vector quantity (i.e. a directional quantity) related to rotation or spin, and is equal to the mass of the particle multiplied by the cross product of the position vector of the particle with its velocity vector. The angular momentum of a system of particles is the sum of that of the particles within it. For each nucleus there are $2I + 1$ allowed spin states with integral differences ranging from $-I$ to $+ I$, i.e. $+I$, $(I - 1) - (-I + 1)$, $-I$.

For example, $I = \frac{1}{2}$ for hydrogen and allowed spin states are $+\frac{1}{2}$ and $-\frac{1}{2}$.

For chlorine atom $I = \frac{3}{2}$, therefore, allowed spin states are $+\frac{3}{2}, +\frac{1}{2}, -\frac{1}{2}, -\frac{3}{2}$.

These spin states have equal amount of energy (degenerated) in the absence of a magnetic field. But when a magnetic field is applied these protons possessing spin and there own magnetic field align themselves either with or opposite to the applied magnetic field. For example, ${}^{1}_{1}H$ atom has $+\frac{1}{2}$ and $-\frac{1}{2}$ spin state. The protons which have spin state of $+\frac{1}{2}$ align themselves with the field (hence comparatively lower energy), and those who have $-\frac{1}{2}$ spin state align themselves opposite to the field (therefore, comparatively higher energy), Figs 11.1 and 11.2. Hence, the external magnetic field has split the spin state into higher and lower level (Fig. 11.3).