Introduction

Chapter 1

Analytical chemistry[1] is a branch of chemistry that deals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts. In practice, quantifying analytes in a complex sample becomes an exercise in problem solving. Traditionally, analytical chemistry has been split into two main types, qualitative and quantitative seeks to establish the presence and amount of a given element or compound in a sample respectively.

Analytical method validation

Validation[2] of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Validation is a basic requirement to ensure quality and reliability of the results for all analytical applications.

Method validation[3] is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods, guidelines from the United States Pharmacopoeia (USP), International Conference on Harmonization (ICH), Food and Drug Administration (FDA) etc., provide a framework for performing such validations.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, determine by means of well-documented experimental studies.

Reasons for Validation

There are two important reasons for validating assays of active pharmaceutical ingredients in the pharmaceutical industry. The first, and by-far the most important, is that assay validation is an integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation. In industry it

would be difficult to confirm that the product being manufactured is uniform and that meet the standards set to assure fitness for use. The varying nature of the differences between the analytical development laboratory and quality control laboratory is a good reason for validation program.

Benefits of Method Validation

A fully validated process may require less in-process control and end-product testing. It deepens the understanding of processes, decrease the risks of processing problems, and thus assure the smooth running of the process.

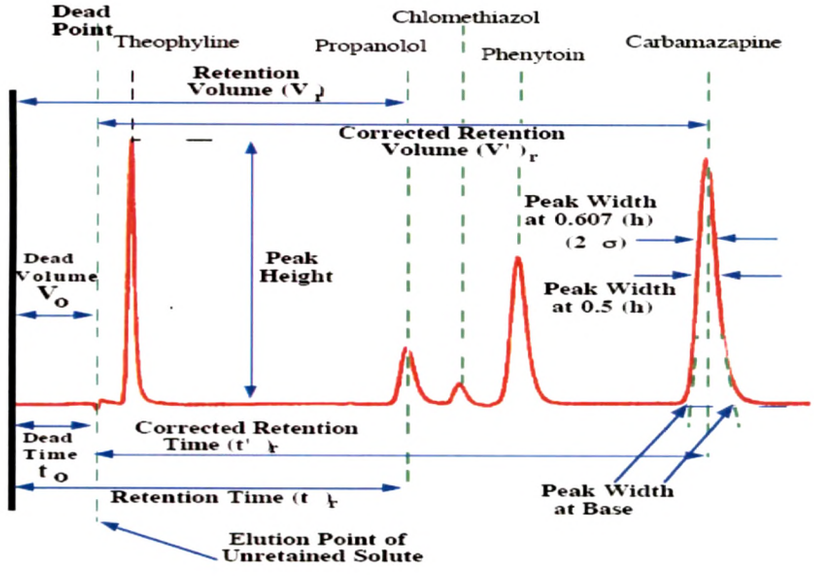
Typical validation characteristics which should be considered are listed below[4]

- Accuracy
- Precision
  - -Repeatability
  - -Reproducibility
- Specificity
- Linearity
- Range
- Detection limit
- Quantitation limit
- Ruggedness
- Robustness etc.,

1.1 High Performance Liquid Chromatography (HPLC): Chromatography was discovered by M. S. Tswett in 1903. Chromatography encompasses a diverse but related group of methods that permit the separation, isolation, identification and quantification of

components in a mixture[5]. HPLC is one mode of chromatography, the most widely used analytical technique. The process occurs as a result of many sorptions-desorption steps during the movement of sample components through the stationary phase. Separation is due to differences in the distribution coefficients of the sample components.

HPLC utilizes a liquid mobile phase to separate the components of a mixture. These components (or analytes) are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture is resolved into its components. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures.



The nomenclature of chromatogram

HPLC measurements are susceptible to variations in analytical conditions, these should be suitably controlled, or a precautionary statement should be included in the method.

One consequence of the evaluation of robustness and ruggedness should be that a series of system suitability parameters is established to ensure that the validity of the analytical method is maintained wherever used. Typical variations are the stability of analytical solutions, different equipment, and different analysts.

Acceptance criteria: The RSD variation of the results ± 2.5%.

System suitability specifications and tests for HPLC[3]

The accuracy and precision of HPLC data collected begin with a well behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose.

1. Capacity factor (k')

$k' = (t_R - t_0) / t_0$

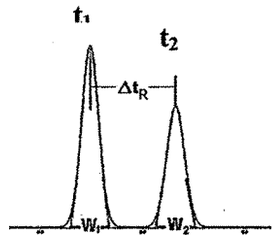
The capacity factor is a measure of the degree of retention of an analyte relative to an unretained peak, where t_R is the retention time for the sample peak and t_o is the retention time for an unretained peak.

Recommendations:

The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

2. Resolution (R_s)

Ability of a column to separate chromatographic peaks. Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase. It can also be expressed in terms of the separation of the apex of two peaks divided by the tangential width average of the peaks.



$R_s = \Delta t_R / 0.5 (W_1 + W_2)$;

Where $\Delta t_R = t_2 - t_1$

For reliable quantitation, well-separated peaks are essential for quantitation.

Recommendations:

Rs of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc) are desirable.

3. Tailing factor (T)

A measure of the symmetry of a peak, given by the following equation where $W_{0.05}$ is the peak width at 5% height and f is the distance from peak front to apex point at 5% height. Ideally, peaks should be Gaussian in shape or totally symmetrical.

$T = W_{0.05} / 2f$

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak. Integrator variables are preset by the analyst for optimum calculation of the area for the peak of interest.

Recommendations:

T of ≤ 2

4. Theoretical plate number / Efficiency (N)

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A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. The most common are shown here, with the ones most sensitive to peak shape shown first:

4-sigma / tangential

$N = 16 (t_R / W)^2 = L / H$

Half height

$N = 5.54 (t_R / W)^2 = L / H$

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram, where t_R is the retention time for the sample peak and W is the peak width.

N is fairly constant for each peak on a chromatogram with a fixed set of operating conditions. H, or HETP, the height equivalent of a theoretical plate, measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include Peak position, particle size in column, flow-rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte.

Recommendations:

The theoretical plate number depends on elution time but in general should be > 2000.

General Recommendation:

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The amount of testing required will depend on the purpose of the test method. For dissolution or release profile test methods using an external standard method, k', T and RSD are minimum recommended system suitability tests. For acceptance, release, stability, or impurities/degradation methods using external or internal

standards, k', T, Rs and RSD are recommended as minimum system suitability testing parameters. In practice, each method submitted for validation should include an appropriate number of system suitability tests defining the necessary characteristics of that system.

Additional tests may be selected at the discretion of the reviewer.

1.2 High Performance Thin Layer Chromatography (HPTLC): The term "thin layer chromatography" was coined by Egon Stahl in Germany in the late 1950's. Stahl's greatest contribution to the field was standardization of materials, procedures and nomenclature and description of selective solvent systems for the resolution of many important compound classes. In present day HPTLC is without doubt one of the most versatile and widely used separation methods in chromatography. Commercially, many sorbents on a variety of backings and mobile phases are now available and allows the handling of a large number of samples in one chromatographic run. It has found a use in a wide range of application areas as the concept of HPTLC is so simple and samples usually require only minimal pretreatment, detecting components at low nanogram sensitivities. In its simplest form, HPTLC costs little, but even including the more sophisticated instrumentation, it still remains less expensive per sample analysis than HPLC. The modern HPTLC has seen a strong move in the direction of plate scanning and video imaging as a means of providing sensitive and reliably accurate results and a more permanent record of chromatogram.[5]

1.3 Spectrophotmetric methods: Several series of reports have been published on the development of derivative spectroscopy (DS). In recent years, the use of DS has become

more practical owing to the increase in the resolving power of the analytical instrumentation and the easier access to microcomputers with appropriate software, which allows the almost instantaneous generation of the derivative spectra. This facility in the collection and treatment of the spectra has allowed analysis of multicomponent mixtures of analytes with strongly overlapping spectra.

The purpose of an analytical process is to measure the concentration of an analyte in a medium. The only relevant question concerning the purpose of measurement is: How well or reliably does it measure? Applying chemometrics to performance characterization of analytical processes leads to a new, comprehensive measurement reliability characterization system. The analytical process should take the responsibility to publicize allowable errors. Current statistical approaches to characterization of analytical processes either do not include measurement reliability or do not address it adequately. The unified, comprehensive chemometric system described here does address measurement reliability[6].

The Process Analytical Technology (PAT) initiative has introduced a number of rapid spectroscopic methods of analysis to the pharmaceutical, chemical, food and related industries, but associated with these methods is a large amount of data to be interpreted. Applications, such as Near Infrared (NIR) Spectroscopy requires statistical methods to be applied to extract the relevant information out of the data. The data analysis methods are collectively referred to as Chemometrics. The principles of chemometrics however extend beyond its application to spectroscopic data, for example, analyzing chromatographic, electrometric data etc[3]. This is a rapid spectroscopic tool to handle large data. Applying the principles of chemometrics can lead to development of a

powerful, simple, comprehensive system for characterizing measurement reliability of analytical processes.

"Chemometrics is defined as the chemical discipline that uses mathematical and statistical methods to design or select optimal measurement procedures, experiments and to provide maximum chemical information by analyzing chemical data. Chemometrics is the chemical discipline that uses mathematical and statistical methods for the obtention in the optimal way of relevant information on material systems"[7].

1.3.1 Simultaneous equation method (Vierodt's method): Concentration of several components present in the same mixture can be determined by solving a set of simultaneous equations even if their spectra overlap. If Beer's law is followed, these equations are linear, Cramer's Rule is a handy way to solve for just one of the variables without having to solve the whole variables in the system.

$A_1 = a_1 x + b_1 y \ldots$ (1) and $A_2 = a_2 x + b_2 y \ldots$ (2)

Where A_1 and A_2 are the absorbances, a_1 and a_2 are the absorptivity values of component x, b_1 and b_2 are the absorptivity values of component y at wavelengths λ_1 and λ_2 respectively. Assuming that $a_1 b_2 - a_2 b_1$ is not equal to zero, solve the above equation for x to obtain.

$$x = (c_1 b_2 - c_2 b_1) / (a_1 b_2 - a_2 b_1)$$

We can use similar steps to eliminate x and solve for y to obtain.

$$y = (a_1 c_2 - a_2 c_1) / (a_1 b_2 - a_2 b_1)$$

1.3.2 The absorption ratio method (Q- Analysis): The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g., two dilutions of the same substance give the same absorbance ratio A_1 / A_2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths. One being the λ_{max} of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., an iso-absorptive point.

1.3.3 Zero-crossing derivative spectra method: This is the most common procedure for the simultaneous determination of binary mixtures of their overlapping spectra. By measuring the derivative values for mixture (zero-crossing wavelength of one analyte) one can avoid the interference by another analyte vice-versa and the concentration of analytes can be determined without any separation step. However this necessitates the selection of zero-crossing wavelength for the measurements[8].

1.3.4 Derivative spectrophotometric method: is useful means of resolving two overlapping spectra and eliminating matrix interference due to an indistinct shoulder on side of an absorption bands. It involves conversion of normal spectrum [$A = f(\lambda)$] to its first [$dA / d\lambda = f(\lambda)$], second [$d^2A / d\lambda^2 = f(\lambda)$] and higher derivatives spectra where the amplitude in the derivative spectrum is proportional to the concentration of the analyte provided that Beer's law is obeyed by the fundamental spectrum.

1.3.5 Ratio derivative spectra (Derivative quotient spectra): This permits the use of the wavelengths corresponding to maximum/minimum and also the use of the distance between consecutive maximum and minimum. The method was based on dividing the spectrum for a mixture into the standard spectra for each of the analyses and driving the quotient to obtain a spectrum that is independent of analyte concentration used as a divisor. The use of standardized spectra as divisor minimizes experimental errors and background noise[9]. An accurate choice of standard divisor and working wavelengths is fundamental for several reasons[10-12].

Easy measurements on separate peaks, higher values of the analytical signals and no need to work only at zero-crossing points are advantages for ratio derivative spectroscopy in comparison of lot of maxima and minima in ratio spectra derivative data was another advantage, since these compounds in the presence of other active compounds and excipients that possibly interfered with the assay[13].

Resolution of binary and ternary mixtures by applying derivative ratio spectra (Derivative quotient spectra)

$$A_{M,\lambda_1} = \varepsilon_{A,\lambda_1} C_A + \varepsilon_{B,\lambda_1} C_B + \varepsilon_{C,\lambda_1} C_C$$

Where, A_M,λ_1 is the absorbance value of the mixture at wavelength λ_1, ε_A,λ_1, ε_B,λ_1 and ε_C,λ_1 are the molar absorptivities of *A*, *B* and *C* at λ_1, C_A, C_B *and* C_C are the molar concentrations of *A*, *B* and *C* respectively.

$$A^0{}_{A,\lambda} = \varepsilon^0{}_{A,\lambda_1} C^0{}_A$$

We obtain

$$\frac{A_{M,\lambda_1}}{A^0{}_{A,\lambda_1}} = \frac{C_A}{C^0{}_A} + \frac{C_B \varepsilon_{B,\lambda_1}}{C^0{}_A \varepsilon_{A,\lambda_1}} + \frac{C_C \varepsilon_{C,\lambda_1}}{C^0{}_A \varepsilon_{A,\lambda_1}}$$

$$\frac{d}{d\lambda}\frac{A_{M,\lambda_1}}{A^0{}_{A,\lambda_1}} = \frac{C_B}{C^0{}_A}\frac{d}{d\lambda}\left[\frac{\varepsilon_{B,\lambda_1}}{\varepsilon_{A,\lambda_1}}\right] + \frac{C_C}{C^0{}_A}\frac{d}{d\lambda}\left[\frac{\varepsilon_{C,\lambda_1}}{\varepsilon_{A,\lambda_1}}\right]$$

$$\frac{d}{d\lambda}\frac{A_{M,\lambda_1}}{A^0{}_{A,\lambda_1}} = \frac{C_B}{C^0{}_A}\frac{d}{d\lambda}\left[\frac{\varepsilon_{B,\lambda_1}}{\varepsilon_{A,\lambda_1}}\right]$$

1.4 Chemometrics

Chemometrics[14-22]are the chemical discipline that uses mathematical and statistical methods for the obtention in the optimal way of relevant information on material systems. The chemometric generally presumed that there is a linear relationship between digitized data and component concentration. These methods have a calibration step followed by validation (with validation samples independently prepared) and prediction (new samples) step in which the results of calibration step are used to estimate the component concentration from unknown sample spectrum. There are four chemometric methods, namely:

A). Classical least squares (CLS)

B). Inverse least squares (ILS)

C) Principle component regression (PCR) and

D) Partial least squares (PLS) calibrations.

The chemometric methods have many of full spectrum advantages. These methods provide additional advantages where calibration can be performed by ignoring the concentration of all other components except the analyte of interest. The methods do not require any derivatization, prior separation or sample pretreatment. These methods have been successfully applied to the quantitative analysis in spectrophotometric, chromatographic and electrochemical data.

Application of Chemometrics on Spectral Data:

1.4.1 Classical least squares (CLS): This method assumes Beer's law model with the absorbance at each frequency being proportional to the component concentrations. In matrix notation, Beer's law model for m calibration standards containing l chemical components with the spectra of n digitized absorbencies is given by:

$A = C \times K + E_A$ .., (1)

Where A is the $m \times n$ matrix of absorbance spectra, C is the $m \times l$ matrix of component concentration, K is the $l \times n$ matrix of absorptivity-path length products, and E_A is the $m \times n$ matrix of spectral errors.

K, then represents the matrix of pure component spectra at unit concentration and unit path length. The classical least squares solution to Eq. (1) during calibration is,

$\bar{K} = (C^T C)^{-1} C^T \times A$

Where $\bar{K}$ indicates least- squares estimates of K.

And analysis based on the spectrum a, of unknown components concentration (samples),

$c_0 = (\bar{K}\,\bar{K}^T)^{-1} \bar{K} \times a$

Where, c_0 is vector of predicted concentrations and $\bar{K}^T$ is transpose of the matrix $\bar{K}$.

1.4.2 Inverse least squares (ILS): This method treats that concentration as a function of absorbance. The inverse of Beer's law model for m calibration standards with spectra of n digitized absorbencies is given by

$C = A \times P + Ec$ .., (2)

Where C and A are as before, P is the $n \times l$ matrix of unknown calibration coefficient relating

the l component concentrations of the spectral intensities, and Ec is the $m \times l$ vector of errors.

The inverse least square solution during calibration for P in Eq. (2) is

$$\bar{P} = (A^T A)^{-1} A^T \times C$$

Where $\bar{P}$ represents least-square estimates of P.

During prediction a, the solution for the analyte concentration in unknown sample is simply

$$c_0 = a^T \times \bar{P}$$

Since in ILS the number of frequencies cannot exceed the total number of calibration mixtures used, stepwise multiple linear regressions have been used for the selection of frequencies[15].

1.4.3 Principle component regression (PCR): The original data obtained in absorbencies A and concentrations C of mixtures were transformed by mean–centered and scaled (optional) into A_0 and C_0, respectively.

This model building procedure has two fundamental concepts: Firstly, the computation of eigen values and their eigenvectors corresponding to the covariance square matrix of the A_0. Secondly, by using cross-validation in the calibration step, the optimal principal components (or the eigenvectors) corresponding to the large eigen values is selected. Using the ordinary linear regression $C = a + b \times A$ we calculate the coefficients a and b. Coefficient $b = P \times Q$ where P is the matrix of eigenvectors and Q is C-loadings given by $Q = D \times T^T \times A_0$. Here T^T is the transpose of the score matrix T. D is a diagonal matrix having on the components the inverses of the selected eigen values. Knowing b, we found a using formula $a = C_{mean} - A^T_{mean} \times b$, where C_{mean} represents the mean concentration of

the calibration set and A^T_{mean} is the transpose of the matrix having the entries of mean absorbance values.

The 'pcr' function of the R-software environment (version 2.1.1) package "pls" (http://mevik.net/work/software/pls.html (URL for pls package)) implements the well-known algorithm[22, 23] based on singular value decomposition. This function was used to fit PCR model using the transformed data as input. The 'Leave-one-out' (LOO) CROSS validation method was chosen while fitting the PCR model.

1.4.4 Partial least squares (PLS): The PLS calibration using the orthogonalised PLS algorithm involves simultaneously the independent and the dependent variables on the data compression and decomposition operations resulting into latent variables, $A = TP^T + E$ and $C = UQ^T + F$. The vector, b is given by $b = W (P^TW)^{-1} \times Q$, where W is a PLS weight matrix. By using the linear regression $C = a + b \times A$, the constant a is calculated by $a = C_{mean} - A^T_{mean} \times b$ as in the PCR method, the PLS calibration is used for the estimation of active principle ingredients.

Three chemometric methods other than the ILS have many full spectrum advantages. Thus wavelength selection is seemingly unnecessary, and so all available wavelengths are often used. Stepwise multiple linear regressions have been used for the selection of frequencies in ILS[24]. All the information present in the sample target should be present in the calibration data set. It has been one of the main drawbacks in development studies of multivariate method. However, measurements from spectral wavelengths that are not informative in a model will degrade performance. Original and reconstructed spectra of the calibration matrix were compared in order to select the range of wavelengths. The region, which is best, reconstructed also considered.

Statistical parameter to evaluate the efficiency of chemometric calibration mode

The predictive ability of a calibration model in chemometric methods can be defined in various ways. The most general expression is the standard error of calibration (SEC) and prediction (SEP), which is given by the following equation;

$$\text{SEP (SEC)} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n}}$$

and

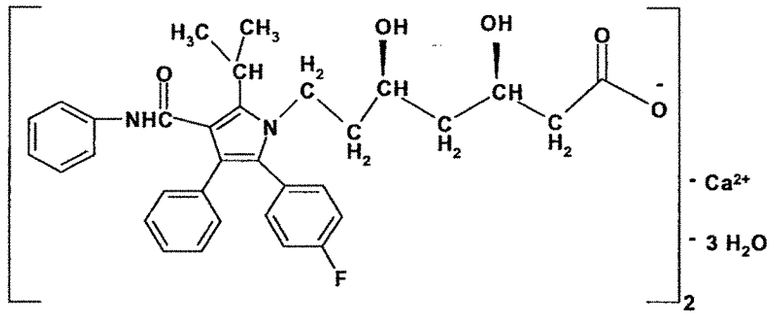
$$\text{PRESS} = \sum_{i=1}^{n}\left(C_i^{Added} - C_i^{Found}\right)^2$$

Here C_i^{Added} is the added concentration of candidate concentrations C_i^{Found} is the predicted concentration and n is the total number of the synthetic mixtures. The chemometric calculations on the resulting data can be realized on various soft wares on which an algorithms to solve the above calculations has to be written. Softwares like MATLAB 8.0, SPSS ver.10, R-Software and others that are available on web sources.

1.5 Drug profile

1.5.1 Atorvastatin

Structural formula:



Systematic (IUPAC) name: [R-(R*, R*)]-2-(4-fluorophenyl)-beta, delta-dihydroxy-5- (1-methylethyl)-3-phenyl-4- [(phenylamino)carbonyl]-1H- pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

Empirical Formula: $(C_{33}H_{34}FN_2O_5)$ $Ca.3H_2O$

Mol. weight: 1209.42 g mol^{-1}

Appearance: A white to off white crystalline powder.

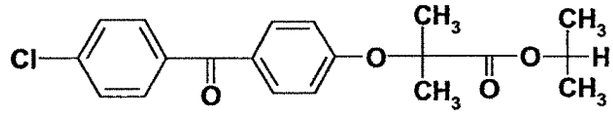
Melting point: 159^0-161^0C

Solubility: Soluble in methanol, Acetonitrile, Slightly soluble in water.

Approved Name: Atorvastatin Calcium

1.5.2 Fenofibrate

Structural Formula :



Systematic (IUPAC) name: 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester

Empirical formula : $C_{20}H_{21}O_4Cl$

Mol. weight: 360. 83 g mol^{-1}

Appearance: white solid which is stable under ordinary conditions

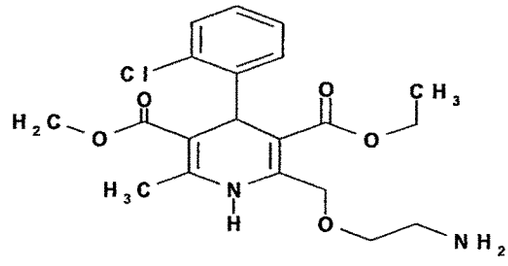
Melting point: 79^0 -82^0C

Solubility: It is insoluble in water

Approved Name: Fenofibrate

1.5.3 Amlodipine Besylate

Structural formula:



Systematic (IUPAC) name: 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate

Empirical formula : $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$

Mol. weight: 567.1 g mol^{-1}

Appearance: white crystalline powder

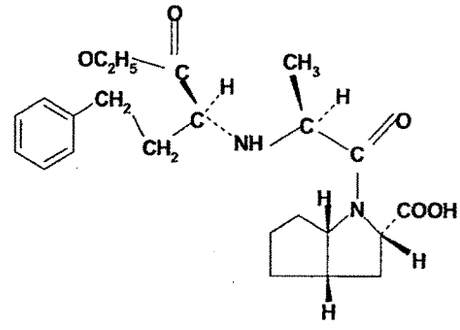
Melting point: 178^0-180^0C

Solubility: It is slightly soluble in water and sparingly soluble in ethanol

Approved Name: Amlodipine Besylate

1.5.4 Ramipril

Structural formula:



Systematic (IUPAC) name: 2-[N-[(S)-1-ethoxy carbonyl-3-phenyl propyl]-L-alanyl]-91S, 3S, 5S)-2-azabicyclo [3, 3, 0] octane-3-carboxylic acid

Empirical formula : $C_{23}H_{32}N_2O_5$

Mol. weight: 416.5 g mol^{-1}

Appearance: A white crystalline powder

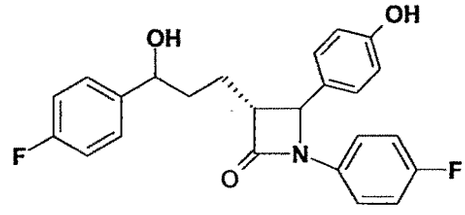
Melting point: 109^0-111^0C

Solubility: Ramipril is slightly soluble in water. Soluble in polar organic solvents and buffered aqueous solutions.

Approved Name: Ramipril

1.5.5 Ezetimibe

Structural formula :



Systematic (IUPAC) name: 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone

Empirical formula : $C_{24}H_{21}F_2NO_3$

Molecular weight: 409.4 g mol^{-1}

Appearance: white, crystalline powder

Melting point: 163°-164^0C

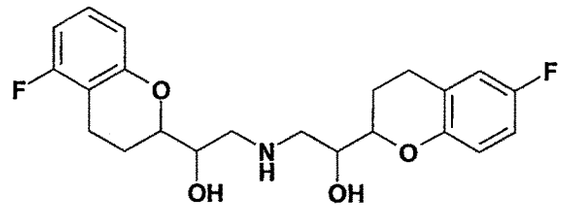
Solubility: freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water

Approved Name: Ezetimibe

1.5.6 Nebivolol

Systematic (IUPAC) name: (+)-[R[s[s[s-(s)]]] α – α –[imino bis(methylene)bis[6-fluoro-3,4-dihydro-2H-1-benzo-pyron-2-methanol]

Structural formula:



Empirical Formula: $C_{22}H_{25}NF_2O_4$

Mol. weight: 405.435 g mol^{-1}

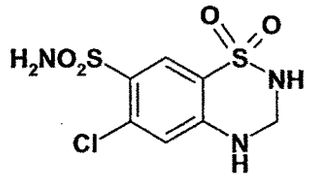
Appearance: A white to off white crystalline powder.

Solubility: Soluble in methanol, sparingly soluble in Acetonitrile.

Approved Name: Nebivolol

1.5.7 Hydrochlorothiazide

Structural Formula :



Systematic (IUPAC) name: 6-chloro-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide

Empirical formula : $C_7H_8ClN_3O_4S_2$

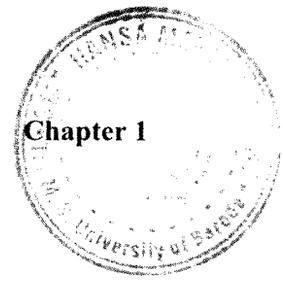
Mol. weight: 297.73 g mol^{-1}

Appearance: A white, or practically white, crystalline powder

Melting point: 273^0-275^0

Solubility: Hydrochlorothiazide is slightly soluble in water. It is soluble in dilute ammonia, dilute aqueous sodium hydroxide and dimethylformamide. It is sparingly soluble in methanol.

Approved Name: Hydrochlorothiazide



Chapter 1

1.6 Biological significance of drugs

1.6.1 Atorvastatin (ATOR)

Atorvastatin (ATOR) is a member of the class of lipid lowering agents called statins. ATOR is potent inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA), the rate limiting enzyme in cholesterol biosynthesis and has been demonstrated to be effective in both cholesterol and triglyceride[25, 26]. Cardiovascular disease (CVD) in particular coronary heart disease (CHD) is the principal cause of morbidity and mortality. Elevated plasma total cholesterol and low density cholesterol levels have been shown repeatedly to be predictive of premature CHD[27]. The statin class of (HMG-CoA) reductase inhibitors represents the most efficient drugs for the treatment of hypercholesterolemia, and they can significantly reduce the morbidity and mortality[28]. ATOR has a longer inhibition of HMG-CoA reductase and they have a better effect than other statins. The evidence Collaborative Atorvastatin Diabetes Study (CARDS) showed major relative risk reduction of cardiovascular events, thus ATOR alters the pathogenesis of CVD rapidly, such that the effect on cardiovascular events is apparent within months of initiation of therapy[29]. Endothelial dysfunction represents a critical early component of organ injury following cardiopulmonary bypass, recent studies demonstrate that the treatment with ATOR is associated with a significant improvement on endothelial function independently of its efficacy on cholesterol levels[30].

1.6.2 Fenofibrate (FENO)

The fibric acid derivative fenofibrate (FENO) is indicated as an adjunct to dietary modification in adults with primary hypercholesterolemia or mixed dyslipidemia (types IIa and IIb hyperlipidemia, Fredrickson classification) to reduce levels of low-density

lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides (TG), and apolipoprotein (apo) B, and to increase levels of high-density lipoprotein cholesterol (HDL-C) and apo A. FENO protects against coronary heart disease not only through its effects on lipid parameters but also by producing alterations in LDL structure and, possibly, alterations in the various hemostatic parameters. Its uricosuric property may prove to be a useful adjunctive attribute[31, 32]. In patients with non-alcoholic fatty liver disease, treatment with FENO is safe and improves metabolic syndrome, glucose and liver tests[33]. FENO seems to act as a weight-stabilizer mainly through its effect on liver metabolism[34-37]. FENO was also found useful as antinociceptive and antiedematogenic[38], anti-inflammatory[39], antioxidant[40], breast cancer[41], atherogenic dyslipidemia[42], diabetic nephropathy[43] and acting as broad-spectrum lipid lowering agent[44].

1.6.3 Ezetimibe (EZET)

A new drug that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. It is approved for primary hypercholesterolemia (heterozygous familial and nonfamilial hypercholesterolemia) as monotherapy or in combination with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). It is also indicated in combination with atorvastatin for homozygous familial hypercholesterolemia and as an adjunct to diet for homozygous sitosterolemia[45-47].

1.6.4 Ramipril (RAMP)

The prodrug for the major metabolite ramiprilat formed by ester hydrolysis, which is a highly active inhibitor of angiotensin-converting enzyme (ACE), thus blocking the conversion of the angiotensin I to angiotensin II, a highly potent vasoconstrictor and thereby leading to a reduction in vasopressor activity and decrease in peripheral vascular

resistance. RAMP is used in the treatment of all forms of hypertension, heart failure and following myocardial infarction to improve survival in patients with clinical evidence of heart failure[48]. Kidney damage (nephropathy) is a common problem seen in hypertensive patients, ACE inhibitors prevents kidney damage but even amongst them RAMP has the best effect in preventing the kidney damage. Results of the study indicated that RAMP significantly decreased the protein output in urine and reduced progression of nephropathy[49]. Acute Infarction Ramipril Efficacy (AIRE) study revealed postulating that RAMP would lengthen the survival of patients after myocardial infarction which has been enhanced by treatment with thrombolytic agents, aspirin and beta-adrenoreceptor blockade[50].

1.6.5 Nebivolol (NEB)[51]

A new highly β_1 selective antagonist with a noradrenergic, nitric oxide potentiating vasodilatory property, might explain its favourable haemodynamic profile which is unique among β-blockers. In addition, nebviolol does not inhibit the increase in the heart rate response to exercise. NEB has been tested successfully in double-blind, placebo-controlled trials in both hypertension and congestive heart failure.

NEB[52] is administered as a racemic mixture of equal proportions of d- and l-enantiomers. As a lipophilic agent, it is metabolized in the liver and transformed in several active metabolites. It is devoid of intrinsic sympathomimetic or membrane stabilizing activity. Several comparative trials demonstrated an antihypertensive activity that was similar or slightly superior to that observed with various other reference antihypertensive agents. Various studies reported that the drug has a good clinical and biological tolerance profile.

Therefore, NEB may be recommended as an alternative first-line treatment option for the management of patients with mild to moderate essential hypertension.

1.6.6 Hydrochlorothiazide (HCTZ) [23]

The diuretic HCTZ works in the far end (distal) part of the kidney tubules to increase the amount of salt that is removed from the body in the urine. In a low dose of 12.5 to 25 mg per day, this diuretic may improve the blood pressure-lowering effects of other anti-hypertensive drugs. More frequently, however, low doses of HCTZ are used in combination with other anti-hypertensive medications to enhance the effect of the other medications. The mechanism of the antihypertensive effect of thiazides is unknown. HCTZ does not usually affect normal blood pressure.

1.7 Rationale of combination

1.7.1 ATOR and FENO

Atherosclerotic vascular diseases (AVD) are a major cause of morbidity and mortality. Combined hyperlipidemia (CH) is one of the consisting features of metabolic syndrome together with visceral obesity, impaired glucose tolerance and hypertension which are increasingly prevalent factors for premature heart disease and its treatment is troublesome.[53-55] Combination of therapeutic modalities from different classes of agents can be effective, well tolerated and safe in most hard-to-treat patients.[56] Atorvastatin and Fenofibrate combination has a highly beneficial effect on all lipid parameters and plasma fibrinogen. It improved patients AVD risk status significantly more in comparison to each drug alone.[57] Atorvastatin is more effective in reduction of total cholesterol level where as fenofibrate is more efficient in reduction of triglycerides. The combination had beneficial effects on oxidative stress and vascular reactivity in hyperlipidemia.[58-60]

Combination therapy is safe and has beneficial additive effects on endothelial function in patients with combined hyperlipidemia[61].

1.7.2 ATOR and AMLO

Hypertension and dyslipidemia are two of the most commonly co-occurring cardiovascular risk factors that frequently are comorbid. Patients with concurrent hypertension and dyslipidemia are at high risk of cardiovascular disease, yet most patients do not achieve goals both for blood pressure and low-density lipoprotein-cholesterol (LDL-C) [62-64]. Combined treatment of statins and calcium channel blockers has been suggested to be superior to atorvastatin therapy alone [65]. Co administration of AMLO and ATOR is efficacious at reducing blood pressure and improving the lipid profile [66].

1.7.3 ATOR and RAMP

RAMP is prodrug for the major metabolite ramiprilat formed by ester hydrolysis which is highly active inhibitor of (ACE-I)[48]. ATOR belongs to the category of statins, which inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Synthesis of mevalonic acid is impeded, which is a rate-limiting step in cholesterol biosynthesis. This results in a decrease in intracellular cholesterol leading to an increase in the number of synthesis of LDL (low-density lipoproteins) receptors and increased clearance of LDL cholesterol in plasma [67].

Although there have been many advances in the management of cardiovascular diseases (CVD) during the last several years, CVD remains the number one cause for morbidity and mortality. The pathophysiology of CVD reveals RAAS (Renin-angiotensin system)

and dyslipidaemia play an important role in the genesis and progression of CVD risk that is endothelial dysfunction [68]. Also, coronary microcirculation abnormalities have been shown to play a key role in patients found to have chest pain as well angiographically normal epicardial vessels (cardiac syndrome X, SX). This in turn may induce inflammatory and proliferative changes in the vessel wall, which could lead to microvascular dysfunction. Intervention with antioxidant agents such as 3-hydroxy-3-methyl glutaryl coenzyme-A reductase inhibitors (statin), and angiotensin-converting enzyme (ACE-I) have shown to counteract reactive oxygen species production and improve endothelial function in coronary artery disease. Treatment with RAMP plus ATOR resulted in improvements in endothelial function and quality of life of patients with SX [69]. The use of ACE-I and statins has a great impact on endothelial dysfunction and experimental atherosclerosis, the use of both agents may act synergistically in the prevention and treatment of atherosclerosis [70].

1.7.4 ATOR and EZET

Ezetimibe is a novel cholesterol absorption inhibitor that prevents the absorption of dietary and biliary cholesterol in the intestine without affecting the absorption of triglycerides or fat-soluble vitamins, whereas statins inhibit cholesterol production primarily in the liver. This results in a decrease in intracellular cholesterol leading to an increase in the number of synthesis of LDL (low-density lipoproteins) receptors and increased clearance of LDL cholesterol in plasma[71].

When co-administered with ATOR, EZET provided significant incremental reductions in LDL-cholesterol and triglycerides and increases in HDL- cholesterol. Coadministration of ezetimibe and atorvastatin offers a well-tolerated and highly efficacious new treatment

option for patients with hypercholesterolemia[72-74]. It is very important to note that co-administration of EZET and ATOR in renal transplant patients is well-tolerated and effective in reducing important cardiovascular risk factors[75].

1.7.5 NEB and HCTZ

NEB exerts its actions by exhibiting a high selectivity for β1-adrenergic receptors and also by reducing the peripheral vascular resistance by modulating nitric oxide release. The ability of Nebivolol to enhance release of endothelium-derived nitric oxide may have significant clinical implications for the use of this agent in the treatment of hypertension and CVD. Nebivolol has also been shown to significantly reduce morbidity and mortality in a large population of elderly patients with chronic heart failure, independent of left ventricular ejection fraction[76-78]. HCTZ is one of the oldest thiazide diuretics. HCTZ may safely be used to improve ACE inhibition therapy[79]. More recently, NEB has been marketed in combination with HCTZ in tablets. Nebivolol and hydrochlorothiazide were well tolerated. The oral administration of NEB with HCTZ has been found to be more effective than either drug alone in the treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy and effective in reducing clinic and 24-h ambulatory blood pressure in patients with ambulatory hypertension[80].

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