RESULTS AND DISCUSSION

(Method 1, Method 2, Method 3 and Method 4)

Preface:

Four methods for the quantitization of some antihypertensive agents are reported in the presented study. The selected drug combinations were Losartan potassium-Enalapril maleate, Olmesartan medoxomil-Ramipril, Ramipril-Amlodipine besylate and Olmesartan-Amlodipine besylate. The described methods offer precise and accurate results for the simultaneously quantitization of these drug combinations in synthetic bulk drug mixtures and commercial multicomponent tablet formulations devoid of previous extraction and are applied easily for the regular analytical investigations. The methods are also simple, rapid and economical methods which give reproducible results on the instrument used. The reported method is an economical method in which only Double distilled water and Methanol (95%) is used as the solvents and does not require the use of costly reagents. These proposed methods may be applied for the analytical investigation of these drug combinations in bulk drugs and tablet dose forms devoid of the interfering of each other and also additives with a significant and comparative correctness and exactness with the reported methods. Results and discussions for all the newly developed methods is discussed in the following sections separately for different combination with respect to the scheme followed for the method development, results obtained, data about bulk drug analysis and analysis of tablet dose forms, validation results etc.

The binary combinations of some antihypertensive agents which are recently approved as formulation in India were selected for the present study. The multicomponent binary formulations are

- Losartan Potassium and Enalapril maleate
- Olmesartan medoxomil and Ramipril
- Ramipril and Amlodipine besylate
- Olmesartan medoxomil and Amlodipine besylate
The marketed formulations i.e. tablet solid dose forms containing these multicomponent combinations were also studies to prove the applicability of the novel methods for their routine use.

1. Results and Discussion for the first method (Simultaneous determination of Losartan Potassium and Enalapril Maleate):

Results and Discussion:

The standard solutions of Losartan potassium and Enalapril maleate in distilled water (10µg/ml each) subjected to a scan individually at the series of wave-lengths of 200nm to 300nm at zero order and the derivative spectra were taken at N=3 using Shimadzu 1700 spectronic UV-Visible spectrophotometer. Zero crossing point of Losartan potassium was found to be at 205nm and 245nm. Zero crossing point for Enalapril maleate was found to be at 209nm and 250nm. Therefore, 245nm was selected as zero crossing point of Losartan potassium and 209nm was selected as zero crossing point of Enalapril maleate for the present study. Losartan potassium has zero crossing point at 245 nm and Enalapril maleate can be determined at 245nm. Similarly, Enalapril maleate has zero crossing point at 209nm and Losartan potassium can be determined at 209nm. Figure 2[4]. The calibration curve of Losartan potassium was found to be linear at 209nm and the calibration curve of Enalapril maleate was found to be linear at 245nm. Figure 2[8] and 2[16]. There for, it was clear that Losartan potassium and Enalapril maleate can be determined in presence of each other with no intervention of any irrelevant substance in multicomponent combination pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample and concentrations were estimated. Then the technique was subjected to the assay of tablets in marketed dosage forms and satisfactory results were attained within the acceptable limits as per the content of the label claim for Losartan Potassium and Enalapril maleate respectively. Tables 2[4] and 2[5].
The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Losartan and Enalapril was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through intra day and interday precision studies, where the samples were subjected to changed conditions other than optimized parameters. The following discussion is the scheme for validation for the present combination of Olmesartan and Amlodipine besylate in their multicomponent dose forms investigated by First derivatization ultraviolet spectrophotometric method.

Once the ideal technique without interferences is selected for the analysis of medicinal formulations, which is most suitable one, the analysis should be performed at least in duplicate but preferably in triplicate. A simple calculation is converted into information through experimental data obtained which is the reflection of analytical sample to be determined. The results obtained through practical experiments will be always associated with a level of uncertainty as it is true for every physic-chemical measurement. Because of this reason, it is always obligatory to establish the magnitude of this uncertainty to turn the data into meaningful results of the analysis can be presented. For this reason it becomes necessary to validate the developed method and prove the ability of the particular technique for the correct analysis of sample under investigation.

Analytical validation of a method is the practice used to prove the ability of an analytical method in use for a particular trial whether appropriate for its proposed application. Outcomes of the validation of a method are capable of being used to evaluate the excellence, steadiness and constancy of the results of an analysis; it is an essential component of every quality analytical performance. The choice of validation of an analytical method is based on many considerations, such as chemical properties of the analyte and its concentration sample matrix, the time and cost of the analysis, type of measurement i.e., quantitative or qualititative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes present in the sample. A non validated method leads to wrong
interpretations and responsible for the introduction of systematic errors, in turn making the measurement inaccurate and non precise. Methods of analysis need to be validated or re-validated prior to their regular application; at any time the process is altered for which the technique has been validated (for example, a device with diverse characteristics or samples with a diverse matrix); and at any occasion the course of action is distorted and the transformation is exterior the original aptitude of the method. Method validation has established significant notice in the text and from industrialized committees and regulatory authorities. Analysis technique development and validation has an important role in the discovery of new drugs, advancement and production of pharmaceutical substances and their dosage forms. Development of analytical methods is the route of providing an analytical technique for the measurement of the concentration of an active pharmaceutical ingredient in a definite bulk drug or formulated dosage form, which provides easy measures to be used to confirm that, an analytical system, correctly and without fail will provide a dependable quantitative analysis of a pharmaceutically active ingredient in a formulated product. Validation of an analytical method is crucial for the development of methods for analysis and it is a test for the ability of a developed method, broadly for specificity, linearity, accuracy, precision, range, detections limit, quantizations limit, and robustness. In short, development of analysis methods and validating the developed methods confirm a trustworthy measurement of a pharmaceutical formulation that can be carried out accurately.

The technique was validated by principles of ICH guidelines for various parameters including specificity, linearity, accuracy, precision- repeatability (ruggedness), robustness and the results were found to be satisfactory with lower standard deviation and coefficient of variation values within the acceptable limits for Losartan potassium as well as Enalapril maleate in their combined synthetic mixtures and combined dosage forms i.e. marketed tablet formulations for their simultaneous First derivatization UV-spectrophotometric estimation. The method showed specificity in presence of formulation additives, because there was no interfering from the tablets formulation additives. The method was linear, with low deviation values and the regression equation was calculated by the method of least squares (Jeffery et al., 1994b). The method was also accurate, indicated by satisfactory recovery studies at different level of confidence. Intermediate precision studies were carried out by intraday precision and inter-day precision methods by separate analysts and the results were found to be satisfactory demonstrating that, the
The described method offers precise and accurate results for the quantitization of Losartan potassium and Enalapril maleate simultaneously in their synthetic mixture of bulk drugs and commercial multicomponent tablets formulations exclusive of separation and applied without any difficulty for the regular determinations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only distilled water is used as the solvent and does not require the use of costly reagents. This proposed method is competent of being used for the quantitization of Losartan potassium and Enalapril maleate drugs in bulk and tablet dose forms devoid of the interfering of additives with a significant and comparative correctness and exactness with the reported methods. This newly developed method has the advantages over the previously reported methods because, present methods is economical than the two High performance LC methods (Dhole, Khedekar & Amnekar, 2012) and more accurate than the UV-spectrophotometric technique of simultaneous equation (Shrivastava & Gupta, 2011a; Becket & Stenlake, 1997c).

The percentage standard deviation values show that the proposed method provides acceptable variation of Losartan potassium and Enalapril maleate. The standard deviation percentages of proposed technique is within the acceptable limits for Losartan potassium and Enalapril maleate shows the competence of the technique to stay unchanged by minute and purposeful changes in the system restraints and assures its consistency in regular routine application.

The reported method is an ideal method for the routine investigation of the stated combination of drugs. It offers accuracy, precision, sensitivity, selectivity, applicability for routine purposes and has many advantages over zero order spectrophotometric methods. The reported method is simple and rapid method with the conversion of zero order absorption spectrum to first order derivative spectrum automatically with the help of instrumental processing of spectrum.
The drugs were subjected to analysis after getting appropriate dilutions of standard solutions, applied on both bulk drugs and formulations availed from market. The authors claim that the method shows linearity in the employed range with satisfactory accuracy and precision. Quantification of drugs simultaneously in combined preparations is generally accomplished by separating the contents using chromatographical techniques like High Performance Liquid Chromatography, Gas Chromatography and High Performance Thin Layer Chromatography etc. All these techniques provide good accuracy and precision with ideal repeatability, except the expenditure of investigation, which is fairly costly due to high-price instrumentations, reagents and skillfulness. For this reason it is worthwhile to investigate simplest and economically effective methods for any determination including simultaneously quanitization of medicines for conventional tests of formulations. Spectrometric methods of analyses fulfill such requisite wherever the investigation of the multicomponent simultaneously with drugs in combination dosage forms is able to be accepted with comparable efficiency as that of modern chromatographical techniques with significant economical advantages. The development of new products for example, pharmaceutical formulations which may be single component drugs or mixtures rather than the pure materials, i.e. additives, excipients and formulating agents, it is very much desirable to find out the chemical contents of the combination which exhibits the most selective distinctiveness for the reason for which the substance is developed. Chemical purity means freedom from unknown substance. A condition of complete purity is practically unattainable, but may be advanced as narrowly as preferred, afforded enough concern is taken throughout the manufacturing procedure. Still, the lofty expenses attendant upon the achievement of the utmost standards of purity may make the method cost-effectively unsound, so that in application it is a lot essential to thump a balance so as to find a product at reasonable expenditure, which is adequately pure for all pharmaceutical purposes. Thus it would be unfeasible to set down standards for drugs and medicinal substances which would offer for the total nonexistence of even any one impurity. The standards insisted of compounds for medicinal or pharmaceutical uses are resoluted by a variety of aspects which consist of the probably to occur as a consequence of all familiar techniques of production. The crucial decisive factor is safety for use; scrupulous consideration is intended towards foreign substances or impurities which may be harmful or which are capable of incorporating chemical interference when the matter is formulated i.e. the compounded to give a medicinal preparation in a form suitable for
administration. The broad-spectrum constancy of the material is also vital; thus if chemically unsteady, hygroscopic or on the other hand, be supposed to render it fluoresce, then standards have to be so accustomed that substances stored up with sensible care and for reasonable phase of time will still fulfills with the mandatory necessities. Standards must safeguard against the probability of either inadvertent contamination or deliberate defilement of pharmaceutical compounds or formulations.

Based on the above discussion, aim of the present study was an effort for the development of quantitative analytical techniques for the quantitative estimation of some selected combinations of antihypertensive drugs present in their synthetic bulk mixtures and multicomponent formulations for cost effective routine analysis like dissolution studies, determination of drugs in biological fluids, simultaneous release studies, and simultaneous kinetic studies etc.

The advantage chief of the above work is its simplicity, because the instrument described is easy to handle. The other advantage is its applicability for the routine analysis for various routine investigations like dissolutions studies, rate determinations studies, release studies, Pharmacokinetic studies, bioavailability studies and other common day to day evaluations. Another application of this technique is its cost-effectiveness and it is the primary advantage over high performance liquid chromatographic methods of analytical investigation. The method employs distilled water as the only solvent and no other reagent is required. The method utilizes very limited number of apparatus, i.e. routine laboratory glasswares used for dilutions purposes whereas the chromatographic techniques demand costly reagents, solvents and chemicals.

2. Results and Discussion for the Second method (Simultaneous determination of Olmesartan Medoxomil and Ramipril):

Results and Discussion:

The standard solutions of Olmesartan medoxomil and Ramipril in Methanol 95% (10µg/ml each)
subjected to a scan individually in the wave-length series of 200nm to 300nm at zero order and the derivative spectra were taken using Elico: SL-160 double beam UV-vis. Spectrophotometric instrument. Zero crossing point of Olmesartan medoxomil was found to be at 235.4nm Zero crossing point for Ramipril was found to be at 245.4nm. There for, 235.4nm was selected as zero crossing point of Olmesartan medoxomil and 245.4nm was selected as zero crossing point of Ramipril for the present study. Olmesartan medoxomil has zero crossing point at 235.4nm and Ramipril can be determined at 235.4nm. Similarly, Ramipril has zero crossing point at 245.4nm and Olmesartan medoxomil can be determined at 245.4nm. Figure 3[3] The linearity graph of Olmesartan medoxomil was found to be a straight line at 245.4nm and the calibration curve of Ramipril was observed as linear at 235.4nm. Figures 3[8] to 3[11]. There for, it was clear that Olmesartan medoxomil and Ramipril can be determined in presence of each other with no significant intervention of additional components in their multicomponent combination formulations.

With the intention of determining the applicability of the developed scheme for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample and concentrations were estimated. Then the process was used to perform the assay of tablets in marketed tablet dosage forms and satisfactory results were attained within the acceptable limits as per the content of the label claim for Olmesartan medoxomil and Ramipril respectively. Tables 3[4] and 3[5].

The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Losartan and Enalapril was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through intra day and interday precision studies, where the samples were subjected to changed conditions other than optimized parameters. The following discussion is the scheme for validation for the present combination of Olmesartan and Amlodipine besylate in their multicomponent dose forms investigated by First derivatization ultraviolet spectrophotometric method.
Once the ideal technique without interferences is selected for the analysis of medicinal formulations, which is most suitable one, the analysis should be performed at least in duplicate but preferably in triplicate. A simple calculation is converted into information through experimental data obtained which is the reflection of analytical sample to be determined. The results obtained through practical experiments will be always associated with a level of uncertainty as it is true for every physic-chemical measurement. Because of this reason, it is always obligatory to establish the magnitude of this uncertainty to turn the data into meaningful results of the analysis can be presented. For this reason it becomes necessary to validate the developed method and prove the ability of the particular technique for the correct analysis of sample under investigation.

Analytical validation of a method is the practice used to prove the ability of an analytical method in use for a particular trial whether appropriate for its proposed application. Outcomes of the validation of a method are capable of being used to evaluate the excellence, steadiness and constancy of the results of an analysis; it is an essential component of every quality analytical performance. The choice of validation of an analytical method is based on many considerations, such as chemical properties of the analyte and its concentration sample matrix, the time and cost of the analysis, type of measurement i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes present in the sample. A non validated method leads to wrong interpretations and responsible for the introduction of systematic errors, in turn making the measurement inaccurate and non precise. Methods of analysis need to be validated or re-validated prior to their regular application; at any time the process is altered for which the technique has been validated (for example, a device with diverse characteristics or samples with a diverse matrix); and at any occasion the course of action is distorted and the transformation is exterior the original aptitude of the method. Method validation has established significant notice in the text and from industrialized committees and regulatory authorities. Analysis technique development and validation has an important role in the discovery of new drugs, advancement and production of pharmaceutical substances and their dosage forms. Development of analytical methods is the route of providing an analytical technique for the measurement of the concentration of an active pharmaceutical ingredient in a definite bulk drug or formulated dosage
form, which provides easy measures to be used to confirm that, an analytical system, correctly and without fail will provide a dependable quantitative analysis of a pharmaceutically active ingredient in a formulated product. Validation of an analytical method is crucial for the development of methods for analysis and it is a test for the ability of a developed method, broadly for specificity, linearity, accuracy, precision, range, detections limit, quantizations limit, and robustness. In short, development of analysis methods and validating the developed methods confirm a trustworthy measurement of a pharmaceutical formulation that can be carried out accurately.

The technique was validated by principles of ICH guidelines for various parameters including specificity, linearity, accuracy, precision- repeatability (ruggedness), robustness and the results were found to be satisfactory with lower standard deviation and coefficient of variation values within the acceptable limits for Olmesartan medoxomil as well as Ramipril in their combined synthetic mixtures and combined dosage forms i.e. marketed tablet formulations for their simultaneous First derivatization UV-spectrophotometric estimation. The method showed specificity in presence of formulation additives, since no interfering was observed from the tablet formulation additives. The method was linear, with low deviation values and the regression equation was calculated by the method of least squares (Jeffery et al., 1994b). The method was also accurate, indicated by satisfactory recovery studies at different level of confidence. Intermediate precision studies were carried out by intra-day precision and inter-day precision methods by separate analysts and the results were found to be satisfactory signifying that, the scheme was reproducible. The technique was not susceptible to change in the method parameters, because the data obtained were reproducible in different temperature conditions applied at the time of determination of these drug substances with very negligible deviations under the conditions employed. Figure 3[12] and Tables 3[8] to 3[13].

The described method offer precise and accurate results for the simultaneously quantitization of Olmesartan-medoxomil and Ramipril in synthetic drugs in bulk mixtures and commercial multicomponent tablet formulations with no need of separating the components and can be performed with ease for the regular analytical determinations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only Methanol (95%) is used as the solvent
and does not require the use of costly reagents. This proposed method may be applied for the quantitation of Olmesartan medoxomil and Ramipril in drugs in bulk and tablet formulations with no the obstruction of additives with a significant and comparative correctness and exactness with the reported methods.

The described method offer precise and accurate results for the quantitization of Losartan potassium and Enalapril maleate simultaneously in their synthetic mixture of bulk drugs and commercial multicomponent tablets formulations exclusive of separation and applied without any difficulty for the regular determinations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only distilled water is used as the solvent and does not require the use of costly reagents. This proposed method is competent of being used for the quantitization of Olmesartan and Ramipril drugs in bulk and tablet dose forms devoid of the interfering of additives with a significant and comparative correctness and exactness with the reported methods. This newly developed method has the advantages over the previously reported methods because, present method is economical than the two Liquid Chromatographic methods (Dhole, Khedekar & Amnekar, 2012) and more accurate than the UV-spectrophotometric absorption factor correction method (Becket & Stenlake, 1997c).

The percentage RSD i.e. relative standard deviation values prove that the projected technique provides tolerable deviations for the analysis of Olmesartan medoxomil and Ramipril. The percentage standard deviation of proposed scheme is within the accepted limits for Olmesartan medoxomil and Ramipril, which shows the ability of the procedure to stay impassive by minute and planned difference in the process conditions and offers a measure of its reliability in routine application.

The reported method is an ideal method for the routine investigation of the stated combination of drugs. It offers accuracy, precision, sensitivity, selectivity, applicability for routine purposes and has many advantages over zero order spectrophotometric methods. The reported method is simple and rapid method with the conversion of zero order absorption spectrum to first order derivative spectrum automatically with the help of instrumental processing of spectrum.
The drugs were subjected to analysis after getting appropriate dilutions of standard solutions, applied on both bulk drugs and formulations availed from market. The authors claim that the method shows linearity in the employed range with satisfactory accuracy and precision. Quantification of drugs simultaneously in combined preparations is generally accomplished by separating the contents using chromatographical techniques like High Performance Liquid Chromatography, Gas Chromatography and High Performance Thin Layer Chromatography etc. All these techniques provide good accuracy and precision with ideal repeatability, except the expenditure of investigation, which is fairly costly due to high-price instrumentations, reagents and skillfulness. For this reason it is worthwhile to investigate simplest and economically effective methods for any determination including simultaneously quantitization of medicines for conventional tests of formulations. Spectrometric methods of analyses fulfill such requisite wherever the investigation of the multicomponent simultaneously with drugs in combination dosage forms is able to be accepted with comparable efficiency as that of modern chromatographical techniques with significant economical advantages. The development of new products for example, pharmaceutical formulations which may be single component drugs or mixtures rather than the pure materials, i.e. additives, excipients and formulating agents, it is very much desirable to find out the chemical contents of the combination which exhibits the most selective distinctiveness for the reason for which the substance is developed. Chemical purity means freedom from unknown substance. A condition of complete purity is practically unattainable, but may be advanced as narrowly as preferred, afforded enough concern is taken throughout the manufacturing procedure. Still, the lofty expenses attendant upon the achievement of the utmost standards of purity may make the method cost-effectively unsound, so that in application it is a lot essential to thump a balance so as to find a product at reasonable expenditure, which is adequately pure for all pharmaceutical purposes. Thus it would be unfeasible to set down standards for drugs and medicinal substances which would offer for the total nonexistence of even any one impurity. The standards insisted of compounds for medicinal or pharmaceutical uses are resolved by a variety of aspects which consist of the probably to occur as a consequence of all familiar techniques of production. The crucial decisive factor is safety for use; scrupulous consideration is intended towards foreign substances or impurities which may be harmful or which are capable of incorporating chemical interference when the matter is formulated i.e. the compounded to give a medicinal preparation in a form suitable for
administration. The broad-spectrum constancy of the material is also vital; thus if chemically unsteady, hygroscopic or on the other hand, be supposed to render it fluoresce, then standards have to be so accustomed that substances stored up with sensible care and for reasonable phase of time will still fulfills with the mandatory necessities. Standards must safeguard against the probability of either inadvertent contamination or deliberate defilement of pharmaceutical compounds or formulations.

Based on the above discussion, aim of the present study was an effort for the development of quantitative analytical techniques for the quantitative estimation of some selected combinations of antihypertensive drugs present in their synthetic bulk mixtures and multicomponent formulations for cost effective routine analysis like dissolution studies, determination of drugs in biological fluids, simultaneous release studies, and simultaneous kinetic studies etc

The advantage chief of the above work is its simplicity, because the instrument described is easy to handle. The other advantage is its applicability for the routine analysis for various routine investigations like dissolutions studies, rate determinations studies, release studies, Pharmacokinetic studies, bioavailability studies and other evaluations. Another application of this technique is its cost-effectiveness and it is the primary advantage over high performance liquid chromatographic methods of analytical investigation. The method employs methanol as the only solvent and no other reagent is required. The method utilizes very limited number of apparatus, laboratory glasswares used for dilutions purposes whereas the chromatographic techniques demand costly reagents, solvents and chemicals.

3. Results and Discussion for the Third method (Simultaneous determination of Ramipril and Amlodipine Besylate):

Results and Discussion:

The standard solutions of Ramipril and Amlodipine besylate in Methanol 95% (20µg/ml each) taken a scan each independently in the wave-length region of 200nm to 260nm at zero order and
the derivative spectra were taken at N=5 using Shimadzu 1700 spectronic UV-Visible spectrophotometer. Zero crossing point of Ramipril was found to be at 208nm and 258nm. Zero crossing point for Amlodipine besylate was found to be at 211nm, 225nm and 238nm. There for, 258nm was selected as zero crossing point of Ramipril and 225nm was selected as zero crossing point of Amlodipine besylate for the present study. Ramipril has zero crossing point at 258 nm and Amlodipine besylate can be determined at 258nm. Similarly, Amlodipine besylate has zero crossing point at 225nm and Ramipril can be determined at 225nm. (Figure 4.4) The standard graph of Ramipril was observed to be linear at 225nm and the calibration curve of Amlodipine besylate was found to be linear at 258nm. Figure 4[8] and Figure 4[17]. There for, it was clear that Ramipril and Amlodipine besylate can be determined in presence of each other with no obstruction of another component in the multicomponent combination product formulations.

With the aim of determining the practicability of the developed technique on the assay of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample and concentrations were estimated. After that the process was tried for the assessment of tablets in marketed pharmaceuticals and satisfactory results were found within the acceptable limits as per the content of the label claim for Ramipril and Amlodipine besylate correspondingly. Table 4[4] and 4[5]

The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Losartan and Enalapril was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through intra day and interday precision studies, where the samples were subjected to changed conditions other than optimized parameters. The following discussion is the scheme for validation for the present combination of Olmesartan and Amlodipine besylate in their multicomponent dose forms investigated by First derivatization ultraviolet spectrophotometric method.

Once the ideal technique without interferences is selected for the analysis of medicinal formulations, which is most suitable one, the analysis should be performed at least in duplicate
but preferably in triplicate. A simple calculation is converted into information through experimental data obtained which is the reflection of analytical sample to be determined. The results obtained through practical experiments will be always associated with a level of uncertainty as it is true for every physic-chemical measurement. Because of this reason, it is always obligatory to establish the magnitude of this uncertainty to turn the data into meaningful results of the analysis can be presented. For this reason it becomes necessary to validate the developed method and prove the ability of the particular technique for the correct analysis of sample under investigation.

Analytical validation of a method is the practice used to prove the ability of an analytical method in use for a particular trial whether appropriate for its proposed application. Outcomes of the validation of a method are capable of being used to evaluate the excellence, steadiness and constancy of the results of an analysis; it is an essential component of every quality analytical performance. The choice of validation of an analytical method is based on many considerations, such as chemical properties of the analyte and its concentration sample matrix, the time and cost of the analysis, type of measurement i.e., quantitative or qualititative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes present in the sample. A non validated method leads to wrong interpretations and responsible for the introduction of systematic errors, in turn making the measurement inaccurate and non precise. Methods of analysis need to be validated or re-validated prior to their regular application; at any time the process is altered for which the technique has been validated (for example, a device with diverse characteristics or samples with a diverse matrix); and at any occasion the course of action is distorted and the transformation is exterior the original aptitude of the method. Method validation has established significant notice in the text and from industrialized committees and regulatory authorities. Analysis technique development and validation has an important role in the discovery of new drugs, advancement and production of pharmaceutical substances and their dosage forms. Development of analytical methods is the route of providing an analytical technique for the measurement of the concentration of an active pharmaceutical ingredient in a definite bulk drug or formulated dosage form, which provides easy measures to be used to confirm that, an analytical system, correctly and without fail will provide a dependable quantitative analysis of a pharmaceutically active
ingredient in a formulated product. Validation of an analytical method is crucial for the development of methods for analysis and it is a test for the ability of a developed method, broadly for specificity, linearity, accuracy, precision, range, detections limit, quantizations limit, and robustness. In short, development of analysis methods and validating the developed methods confirm a trustworthy measurement of a pharmaceutical formulation that can be carried out accurately.

The technique was validated by principles of ICH guidelines for various parameters including specificity, linearity, accuracy, precision- repeatability (ruggedness), robustness and the results were found to be satisfactory with lower standard deviation and coefficient of variation values within the acceptable limits for Ramipril as well as Amlodipine besylate in their combined synthetic mixtures and combined dosage forms i.e. marketed tablet formulations for their simultaneous First derivatization UV-spectrophotometric estimation. The method showed specificity in presence of formulation additives, because no interfering results from the tablets formulation additives was observed. The method was linear, with low deviation values and the regression equation was calculated by the method of least squares (Jeffery et al., 1994b). The method was also accurate, indicated by satisfactory recovery studies at different level of confidence. Intermediate precision studies were carried out by intra-day precision and inter-day precision methods by individual analysts and the results were found to be satisfactory demonstrating the excellent repeatability of the method. The process was not susceptible to change in the method parameters, because the data obtained were reproducible in different temperature conditions applied at the time of determination of these drug substances with very negligible deviations under the conditions employed. Figure 4[7] to 4[24] and Tables 4[8] to 4[13].

The described method offers precise and accurate results for the simultaneous investigations of Ramipril and Amlodipine besylate in synthetic drugs in bulk mixtures and commercially available multi-component tablet dosage forms with no need to separating of components and practiced with ease for the custom quantitative investigations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only Methanol (95%) is used as the solvent and does not require the use of costly reagents. This proposed method may be used
for the analytical determinations of Ramipril and Amlodipine besylate in drugs in bulk and tablet products devoid of the intervention of preparation additives with a significant and comparative correctness and exactness with the reported methods. This newly developed method has the advantages over the previously reported methods because, present method is economical than the four High performance Liquid Chromatographic methods, two TLC methods and more accurate than the UV-spectrophotometric absorption factor correction method (Becket & Stenlake, 1997c; An introduction to analytical method development, 2008).

The percentage RSD i.e. relative standard deviation values show the ability of proposed method to provide agreeable variation of Ramipril and Amlodipine besylate. The percentage standard deviation of proposed scheme is within the acceptable limits for Ramipril and Amlodipine besylate, which shows the capacity of the process remaining unchanged by minute and intentional deviation in the process conditions and its inductiveness of its consistency in regular and routine applications.

The reported method is an ideal method for the routine investigation of the stated combination of drugs. It offers accuracy, precision, sensitivity, selectivity, applicability for routine purposes and has many advantages over zero order spectrophotometric methods. The reported method is simple and rapid method with the conversion of zero order absorption spectrum to first order derivative spectrum automatically with the help of instrumental processing of spectrum.

The drugs were subjected to analysis after getting appropriate dilutions of standard solutions, applied on both bulk drugs and formulations availed from market. The authors claim that the method shows linearity in the employed range with satisfactory accuracy and precision. Quantification of drugs simultaneously in combined preparations is generally accomplished by separating the contents using chromatographical techniques like High Performance Liquid Chromatography, Gas Chromatography and High Performance Thin Layer Chromatography etc. All these techniques provide good accuracy and precision with ideal repeatability, except the expenditure of investigation, which is fairly costly due to high-price instrumentations, reagents and skillfulness. For this reason it is worthwhile to investigate simplest and economically effective methods for any determination including simultaneously quantitization of medicines for conventional tests of formulations. Spectrometric methods of analyses fulfill such requisite
wherever the investigation of the multicomponent simultaneously with drugs in combination dosage forms is able to be accepted with comparable efficiency as that of modern chromatographical techniques with significant economical advantages. The development of new products for example, pharmaceutical formulations which may be single component drugs or mixtures rather than the pure materials, i.e. additives, excipients and formulating agents, it is very much desirable to find out the chemical contents of the combination which exhibits the most selective distinctiveness for the reason for which the substance is developed. Chemical purity means freedom from unknown substance. A condition of complete purity is practically unattainable, but may be advanced as narrowly as preferred, afforded enough concern is taken throughout the manufacturing procedure. Still, the lofty expenses attendant upon the achievement of the utmost standards of purity may make the method cost-effectively unsound, so that in application it is a lot essential to thump a balance so as to find a product at reasonable expenditure, which is adequately pure for all pharmaceutical purposes. Thus it would be unfeasible to set down standards for drugs and medicinal substances which would offer for the total nonexistence of even any one impurity. The standards insisted of compounds for medicinal or pharmaceutical uses are resoluted by a variety of aspects which consist of the probably to occur as a consequence of all familiar techniques of production. The crucial decisive factor is safety for use; scrupulous consideration is intended towards foreign substances or impurities which may be harmful or which are capable of incorporating chemical interference when the matter is formulated i.e. the compounded to give a medicinal preparation in a form suitable for administration. The broad-spectrum constancy of the material is also vital; thus if chemically unsteady, hygroscopic or on the other hand, be supposed to render it fluoresce, then standards have to be so accustomed that substances stored up with sensible care and for reasonable phase of time will still fulfills with the mandatory necessities. Standards must safeguard against the probability of either inadvertent contamination or deliberate defilement of pharmaceutical compounds or formulations.

Based on the above discussion, aim of the present study was an effort for the development of quantitative analytical techniques for the quantitative estimation of some selected combinations of antihypertensive drugs present in their synthetic bulk mixtures and multicomponent formulations for cost effective routine analysis like dissolution studies,
determination of drugs in biological fluids, simultaneous release studies, and simultaneous kinetic studies etc

The advantage chief of the above work is its simplicity, because the instrument described is easy to handle. The other advantage is its applicability for the routine analysis for various routine investigations like dissolutions studies, rate determinations studies, release studies, Pharmacokinetic studies, bioavailability studies and other common day to day evaluations. Another application of this technique is its cost-effectiveness and it is the primary advantage over high performance liquid chromatographic methods of analytical investigation. The method employs methanol as the only solvent and no other reagent is required. The method utilizes very limited number of apparatus, i.e. routine laboratory glasswares used for dilutions purposes whereas the chromatographic techniques demand costly reagents, solvents and chemicals.

4. Results and Discussion for the Fourth method (Simultaneous determination of Olmesartan medoxomil and Amlodipine Besylate):

Results and Discussion:

The standard solutions of Olmesartan medoxomil and Amlodipine besylate in Methanol 95% (10µg/ml each) individually taken a scan in the wave-length range of 230nm to 280nm at zero order and the derivative spectra were taken at N=5 using Shimadzu 1700 spectronic UV-Visible spectrophotometer. Zero crossing point of Amlodipine besylate was found to be at 237nm and Zero crossing point for Olmesartan medoxomil was found to be at 240nm. There for, 240nm was selected as zero crossing point of Olmesartan medoxomil and 237nm was selected as zero crossing point of Amlodipine besylate for the present study. Olmesartan medoxomil has zero cross position at 240nm and Amlodipine besylate can be determined at 240nm. Similarly, Amlodipine besylate has zero cross position at 237nm and Olmesartan medoxomil can be determined at 237nm. Figure 5[4]. The calibration curve of Olmesartan medoxomil was found to be linear at 237nm and the Linearity graph of Amlodipine besylate was observed to be straight line at 240nm. Figures 5[8] and 5[17]. There for, it was clear that Ramipril and Amlodipine
besylate can be determined in presence of each other devoid of any intervention of another component in the multicomponent combination pharmaceutical products.

So as to determine the practicability of the developed process on the assay of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample and concentrations were estimated. After that the scheme was tried for the assessment of tablets in marketed preparations and satisfactory results were observed within the acceptable limits as per the content of the label claim for Olmesartan medoxomil and Amlodipine besylate respectively. Tables 5[4] and 5[5].

The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Losartan and Enalapril was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through intra day and interday precision studies, where the samples were subjected to changed conditions other than optimized parameters. The following discussion is the scheme for validation for the present combination of Olmesartan and Amlodipine besylate in their multicomponent dose forms investigated by First derivatization ultraviolet spectrophotometric method.

Once the ideal technique without interferences is selected for the analysis of medicinal formulations, which is most suitable one, the analysis should be performed at least in duplicate but preferably in triplicate. A simple calculation is converted into information through experimental data obtained which is the reflection of analytical sample to be determined. The results obtained through practical experiments will be always associated with a level of uncertainty as it is true for every physic-chemical measurement. Because of this reason, it is always obligatory to establish the magnitude of this uncertainty to turn the data into meaningful results of the analysis can be presented. For this reason it becomes necessary to validate the developed method and prove the ability of the particular technique for the correct analysis of sample under investigation.
Analytical validation of a method is the practice used to prove the ability of an analytical method in use for a particular trial whether appropriate for its proposed application. Outcomes of the validation of a method are capable of being used to evaluate the excellence, steadiness and constancy of the results of an analysis; it is an essential component of every quality analytical performance. The choice of validation of an analytical method is based on many considerations, such as chemical properties of the analyte and its concentration sample matrix, the time and cost of the analysis, type of measurement i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes present in the sample. A non-validated method leads to wrong interpretations and responsible for the introduction of systematic errors, in turn making the measurement inaccurate and non-precise. Methods of analysis need to be validated or re-validated prior to their regular application; at any time the process is altered for which the technique has been validated (for example, a device with diverse characteristics or samples with a diverse matrix); and at any occasion the course of action is distorted and the transformation is exterior the original aptitude of the method. Method validation has established significant notice in the text and from industrialized committees and regulatory authorities. Analysis technique development and validation has an important role in the discovery of new drugs, advancement and production of pharmaceutical substances and their dosage forms. Development of analytical methods is the route of providing an analytical technique for the measurement of the concentration of an active pharmaceutical ingredient in a definite bulk drug or formulated dosage form, which provides easy measures to be used to confirm that, an analytical system, correctly and without fail will provide a dependable quantitative analysis of a pharmaceutically active ingredient in a formulated product. Validation of an analytical method is crucial for the development of methods for analysis and it is a test for the ability of a developed method, broadly for specificity, linearity, accuracy, precision, range, detections limit, quantizations limit, and robustness. In short, development of analysis methods and validating the developed methods confirm a trustworthy measurement of a pharmaceutical formulation that can be carried out accurately.

The technique was validated by principles of ICH guidelines for various parameters including specificity, linearity, accuracy, precision-repeatability (ruggedness), robustness and the results
were found to be satisfactory with lower standard deviation and coefficient of variation values within the acceptable limits for Olmesartan medoxomil as well as Amlodipine besylate in their combined synthetic mixtures and combined dosage forms i.e. marketed tablet formulations for their simultaneous First derivatization UV-spectrophotometric estimation. The method showed specificity in presence of formulation additives, because no interfering was observed from the tablets dosage form additives. The method was linear, with low deviation values and the regression equation was calculated by the method of least squares (Jeffery et al., 1994b). The method was also accurate, indicated by satisfactory recovery studies at different level of confidence. Intermediate precision studies were carried out by intra-day precision and inter-day precision methods through separate analysts and the results were found to be satisfactory, demonstrating that, the scheme was reproducible. The process was not susceptible to change in the method parameters, because the data obtained were reproducible in different temperature conditions applied at the time of determination of these drug substances with very negligible deviations under the conditions employed. Figures 5[7] to 5[24] and Tables 5[8] to 5[13].

The described method offer precise and accurate results for the analysis of Olmesartan medoxomil and Amlodipine besylate simultaneously in synthetic drug bulk mixtures and commercial multicomponent product formulations exclusive of previous extraction and are used with easiness for the regular investigations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only Methanol (95%) is used as the solvent and does not require the use of costly reagents. This proposed method may be used for the investigation of Olmesartan medoxomil and Amlodipine besylate in bulk drugs and medicinal dose forms devoid of the interfering of additives with a significant and comparative correctness and exactness with the reported methods. This newly developed method has the advantages over the previously reported methods because, present method is economical than the six High performance Liquid Chromatographic methods, HPTLC method and more accurate than the UV-spectrophotometric technique of simultaneous equation, absorption factor correction method, multivariate method and advantage on the two First derivatization spectrophotometric methods (Shrivastava & Gupta, 2011a; Becket & Stenlake, 1997c; Shimadzu.com, n.d.; Jones, Acetonitrile versus Methanol, n.d.; Majors, 2009).
The percentage RSD i.e. relative standard deviation values show that the proposed method provides acceptable variation of Olmesartan medoxomil and Amlodipine besylate. The percentage standard deviation of proposed method is within the acceptable limits for Olmesartan medoxomil and Amlodipine besylate, which shows the ability of the scheme to stay unaltered by little variations in the characteristics and indicates a sign of its consistency in routine application.

The reported method is an ideal method for the routine investigation of the stated combination of drugs. It offers accuracy, precision, sensitivity, selectivity, applicability for routine purposes and has many advantages over zero order spectrophotometric methods. The reported method is simple and rapid method with the conversion of zero order absorption spectrum to first order derivative spectrum automatically with the help of instrumental processing of spectrum.

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