Introduction:

Metformin hydrochloride is 3-(diaminomethylidene)-1,1-dimethylguanidine, an oral anti diabetic medicine that helps control blood sugar levels (Onal A, 2009). Metformin is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function (Setter SM, 2003). Metformin is sometimes used in combination with insulin or other medications but it is not for treating type 1 diabetes.

In October 2006, the U.S. Food and Drug Administration (FDA) approved sitagliptin as monotherapy and as add-on therapy to either of two other types of oral diabetes medications, metformin or thiazolidinediones to improve blood glucose control in patients with type 2 diabetes when diet and exercise are not enough (Daniel D, 2007). In April, 2007 FDA approved the combination product of sitagliptin and metformin for type 2 diabetes. Sitagliptin is (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo-[3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one, an oral anti-diabetic agent that blocks dipeptidyl peptidase-4 (DPP-4) activity (Badyal DK, 2008).

Sitagliptin phosphate, vildagliptin and saxagliptin is an oral antihyperglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or thiazolidinedione) for treatment of diabetes mellitus type 2. Sitagliptin is synthesised in two reactions steps, hydrogenation in order to obtain the stereospecific free base, followed by the preparation of the phosphate monohydrate salt. Finally the active substance is purified by crystallization (Gallwitz B, 2007). While safety is its advantage, efficacy is often challenged as it is often recommended to be combined with other agents like metformin. The most common side effects in studies were upper respiratory tract infection, stuffy or running nose, sore throat, headache and diarrhea (Patel Kishan D, 2010).

Sitagliptin metabolizes the naturally occurring incretin hormones GLP-1 and GIP resulting in enhanced glucose dependent insulin secretion Vishvas G, 2011).

Several UV-spectrophotometry method were reported for the simultaneous estimation of metformin hydrochloride or its derivatives in combination with several drugs (Khan G, 2011). Two novel cyclic metabolite of sitagliptin were identified and characterized by hydrogen/deuterium exchange tandem mass spectrometry and NMR spectroscopy after purification from dog urine (Liu DQ, 2007). Sitagliptin in human urine and haemodialysate was
estimated by using turbulent flow online extraction and tandem mass spectroscopy (Zeng W, 2008). The developed liquid chromatography-tandem mass spectrometry method for the quantification of sitagliptin in human plasma using liquid-liquid extraction (Nirogi R, 2008). Metformin hydrochloride in combination with other drugs were estimated by using derivative spectrophotometry and by RP-HPLC (Sahoo PK, 2008).

The most characteristic feature of the development in the methodology of pharmaceutical and biomedical analysis during the last 25 years is that HPLC became undoubtedly the most important analytical method for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations during the process of their discovery, development and manufacturing. The next stage is the development stage, where HPLC is used to characterize products of the chemical synthesis, by analyzing the active pharmaceutical ingredients (API), their impurities and/or degradation products generated by accelerated aging. The final goal in the discovery stage in HPLC method for drug is to find a new, safe and active chemical entity that will become medication for diseases.

All HPLC methods used for the development of pharmaceuticals and for the determination of their quality have to be validated. The parameters tested throughout the method validation as defined by the ICH, USP and FDA and other health organizations (95-97) are the following (ICH-Q2B, 1996):

**Selectivity And Specificity** are often used interchangeably. The USP monograph defines selectivity of an analytical method as its ability to measure accurately an analyte in the presence of interference.

**Precision** of a method is measured by injecting a series of standards and measuring the variability of the quantitative results. The measured standard deviation can be subdivided into three categories: repeatability, intermediate precision, and reproducibility (or ruggedness).

**Accuracy** of an analytical method is the extent to which test results are close to their true value. It is measured from the result of a quantitative determination of a well characterized known sample.
Limit of detection is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. In chromatography the detection limit is the injected amount that results in a peak height of at least twice or three times as high as the baseline noise level.

Limit of quantitation is the minimum injected amount that gives precise measurements. In chromatography it typically requires peak heights of 10 to 20 times higher than baseline noise at precision of <10-15% RSD between results.

Robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

STRUCTURAL FORMULA OF SITAGLIPTIN:

NOMENCLATURE: (3R)-3-amino-1-[9-(trifluoromethyl)-1,4,7,8 tetrazabicyclo[4.3.0] nona-6,8-di en-4-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

MOLECULAR FORMULA: C₁₆H₁₅F₆N₅O

MOLECULAR MASS: 407.314 g/mol

DESCRIPTION: White or off white crystalline powder.

SOLUBILITY: It is soluble in water and N, N dimethyl formamide, slightly soluble in methanol, soluble in ethanol, acetone and acetonitrile and insoluble in isopropanol and isopropyl acetate.

ANALYTICAL FUNCTIONAL GROUPS: Amino, Trifluorophenyl

Triazolopiperazine and Carbonyl group.
STRUCTURAL FORMULA OF METFORMIN HYDROCHLORIDE:

NOMENCLATURE: N,N-Dimethylimidodicarbonimidic diamide

MOLECULAR FORMULA: C$_4$H$_{11}$N$_5$

MOLECULAR MASS: 129.16 g/mol

DESCRIPTION: White or off white crystalline powder.

SOLUBILITY: It is soluble in water and, slightly soluble in methanol, soluble in ethanol, acetone and acetonitrile and insoluble in acetone and in methylene chloride.