INTRODUCTION
Analytical method development and validation is an important scientific tool for maintaining and monitoring the quality and stability of drug product; for its shelf life or to establish the shelf life. Analytical methods are also intended to establish the identity, purity, physical characteristics and potency of the drugs that used in drug product.

Quality is very important aspect when it comes to the drug product. Pharmaceutical products formulated with more than one drug to meet patients needs. Quality of such product is critical with respect to dissolution, assay and related compounds. It will be monitor using a specific and stability indicting dissolution, assay and related compound method.

Developing an analytical method to determine dissolution, assay and related compounds for such multi drugs product is an analytical challenge. Impurities are unwanted chemical entities of drug product which are process related or degradation impurities. Method can be developed by using forced degradation of drug product. Forced degradation is a degradation of drug product at accelerated conditions akin to acid, base, peroxide, humidity and thermal.

HIV-1 infection:
Worldwide there are approximately 40 million persons living with human immunodeficiency virus-1 (HIV-1 i.e. type of virus) or acquired immunodeficiency syndrome (AIDS). Of these, over 700,000 people are living within the EU, Norway and Iceland.

The goal of antiretroviral therapy for HIV-1 infection is to delay disease progression and increase the duration of survival by achieving maximal and prolonged suppression of HIV-1 replication. The standard of care for treatment involves the use of a combination of antiretroviral agents, typically a combination of at least three active substances, including a non-nucleoside reverse transcriptase inhibitor (NNRTI) and two active substances from the nucleoside reverse transcriptase inhibitor (NRTI)

Fixed-dose combination of three active substances: efavirenz (an NNRTI), emtricitabine (an NRTI), and tenofovir disoproxil (as fumarate, an NRTI). It is intended to provide combination antiretroviral therapy for administration as a single, once-daily tablet for the treatment of HIV-1 infected adults.

The rationale for the development of this fixed-dose combination is that the individual active substances have shown to be potent and selective inhibitors of HIV-1 reverse transcriptase (RT)
and that their combined use is recommended in national and international HIV-1 infection treatment guidelines (e.g. national European guidelines, US guidelines and WHO guidelines)

**Efavirenz** is a synthetic non-nucleoside reverse transcriptase (RT) inhibitor with antiviral activity. Efavirenz binds directly to the human immunodeficiency virus type 1 (HIV-1) RT, an RNA-dependent DNA polymerase, blocking its function in viral DNA replication. In combination with other antiretroviral drugs, this agent has been shown to significantly reduce HIV viral infection, retarding or preventing damage to the immune system and reducing the risk of developing AIDS. Efavirenz induces activity of the cytochrome P450 system, accelerating its own metabolism.

**Molecular Structure:**

![Molecular Structure Diagram](image)

**Formula:** C\(_{14}\)H\(_{9}\)ClF\(_3\)NO\(_2\)

**Molecular Mass:** 315.68 g/mol

Efavirenz is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. Its molecular formula is C\(_{14}\)H\(_{9}\)ClF\(_3\)NO\(_2\). Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.68. It is practically insoluble in water (less than 10 \(\mu\)g/mL).

**Emtricitabine** is a synthetic fluoro derivative of thiacytidine with potent antiviral activity. Emtricitabine is phosphorylated to form emtricitabine 5'-triphosphate within the cell. This metabolite inhibits the activity of human immunodeficiency virus (HIV) reverse transcriptase both by competing with the natural substrate deoxycytidine 5'-triphosphate and by incorporation into viral DNA causing a termination of DNA chain elongation (due to the lack of the essential 3'-OH group).

**Molecular Structure:**

![Molecular Structure Diagram](image)
**Formula:** C$_8$H$_{10}$FN$_3$O$_3$S  
**Molecular Mass:** 247.24 g/mol  
The chemical name of emtricitabine is 5-fluoro-1-(2R,5S)-[2- (hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of a thio analog of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5-position. It has a molecular formula of C$_8$H$_{10}$FN$_3$O$_3$S.

Emtricitabine is a white to off-white crystalline powder with a solubility of approximately 112 mg/mL in water at 25°C.

**Tenofovir Disoproxil Fumarate** is a pro-drug, fumaric acid salt form of tenofovir, a nucleoside reverse transcriptase inhibitor. Tenofovir disoproxil fumarate is prescribed to treat HIV and chronic hepatitis B virus (HBV) in adults.

**Molecular Structure:**

![Molecular Structure](image)

**Formula:** C$_{19}$H$_{30}$N$_5$O$_{10}$P • C$_4$H$_4$O$_4$  
**Molecular Mass:** 635.52 g/mol  
Tenofovir Disoproxil Fumarate is a fumaric acid salt of the bisisopropoxycarbonyloxymethyl ester derivative of tenofovir. The chemical name of tenofovir disoproxil fumarate is 9-[(R)-2[[bis[[((isopropoxycarbonyl)oxy]-methoxy] phosphinyl]methoxy]propyl]adenine fumarate (1:1). It has a molecular formula of C$_{19}$H$_{30}$N$_5$O$_{10}$P • C$_4$H$_4$O$_4$. 