In reverse phase, the stationary phase used will be a nonpolar hydrocarbons, waxy liquids, or bonded hydrocarbons (such as C18, C8, etc.) while mobile phase is a polar solvents or mixtures such as methanol-water or acetonitrile-water, and etc. the most polar component is eluted first while less polar solvent has higher eluent strength (stoye D and Freitag W, 1996).

1.5.2 Validation of HPLC Instrumentation

According to international conference of harmonization guideline (ICH), FDA and USP the operation of HPLC must be validated and maintained cleaned.

1.6 Definition of parameters used in the validation of the analytical HPLC method

(USP, 2007; ICH, 1994).

1.6.1 Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. The precision of an analytical procedure is expressed as the standard deviation.

1.6.2 System Precision

Used to ascertain injection repeatability and system suitability. It ascertains the effectiveness of the operating system as a single system.

1.6.3 Method Precision

Used to ascertain analysis repeatability by evaluating a number of samples containing known amounts of analyte.