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Review Article

Estimation of Immunosuppressants by Liquid Chromatography Tandem Mass Spectrometry in Clinical Laboratories

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Abstract:

Immunosuppressive drugs or Immunosuppressants have contributed significantly to the success of organ transplantation by preventing graft rejection. They inhibit the activity of the immune system by reducing the proliferation and function of cells associated with immune reactions

Key words: clinical laboratories; immune system; therapeutic drug monitoring; immunosuppressive drugs

Introduction

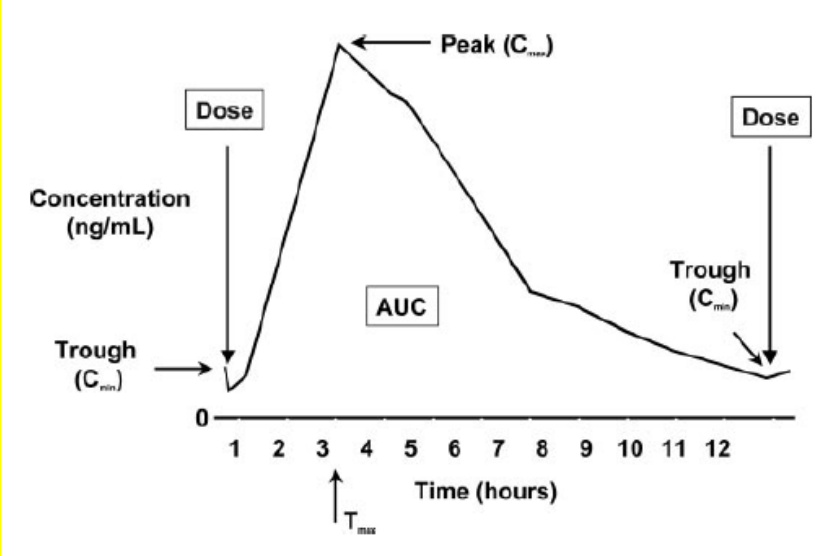
Immunosuppressive drugs or Immunosuppressants have contributed significantly to the success of organ transplantation by preventing graft rejection. They inhibit the activity of the immune system by reducing the proliferation and function of cells associated with immune reactions. They are also used as drugs for autoimmune diseases and other disorders of the immune system.

Indication For Estimation of Immunosuppressants:

Due to their narrow therapeutic index and significant variability in blood concentration between individuals, therapeutic drug monitoring (TDM) of immunosuppressive drugs has been an integral part of organ transplant protocols. Cyclosporine is also commonly used in treatment of aplastic anemia.

To maintain drug concentrations in the therapeutic range and minimize their toxicity or the risk of transplant rejection, regular and periodic monitoring of immunosuppressant drugs is essential [1,2]. Monitoring is also indicated to evaluate patient's adherence or compliance especially in cases of inadequate response.

Concentration of these drugs is lowest (Cmin) just before the dose is taken (trough level) and then rises to a peak level (Cmax) at a certain time after the dose (Tmax). This peak and overall time for drug, remaining in patient's blood stream (AUC) through dosage period differ from patient to patient. Underdosing is associated with an increased risk for rejection, whereas overdosing is associated with pharmacological toxicity (nephrotoxicity, neurotoxicity, hypertension, hyperlipidemia etc.) highlighting the need for periodic monitoring of these drugs with accurate and precise quantification.



Commonly Measured Immunosuppressants for TDM:

Cyclosporine, Tacrolimus (FK506), Everolimus, Sirolimus (Rapamycin) are most commonly measured and monitored immunosuppressants as they are most commonly prescribed drugs following transplant surgeries. All these drugs are inhibitors of either cytokine production or action.

Estimation of Immunosuppressants by Liquid Chromatography Tandem Mass Spectrometry:

Traditional laboratory-based testing for immunosuppressants is usually performed by chemiluminescence immunoassay (CLIA), chemiluminescence microparticle immunoassay (CMIA), radioimmunoassay (RIA), fluorescence polarization immunoassay, Enzyme linked immunoassay (ELISA). However, conventional immunoassays lack analytical specificity due to cross reactivity between these drugs and their metabolites [3]. Moreover, these assays often show interlaboratory variations due to lack of standardization across different platforms and reagents. Assays like ELISA are also prone to manual errors whereas performing RIA based tests is cumbersome and needs regulatory approval and special infrastructure. Another disadvantage of these methods is inability to assay multiple drugs simultaneously leading to reduced operational efficiency. Hence Liquid chromatography tandem mass spectrometry (LC-MS/MS) has emerged as a reliable and "Reference" method for estimation of immunosuppressants.

LC-MS/MS technology combines high performance liquid chromatography (HPLC), a powerful analytical separation technique with mass spectrometry, a highly sensitive detection technique.

Several LC techniques using ultraviolet detection, mass spectrometry or tandem mass spectrometry (TMS) have been developed for the measurement of immunosuppressant drug concentrations [3].

Advantages and Uniqueness of LC-MS/MS:

Efficient use of chromatographic method along with quantifier and qualifier MRM transitions is used in LC-MS/MS to specifically detect actual concentration of target drugs (eliminating metabolites or structural analogues), thereby prevents overestimation.

The current generation of this technology is capable of reliably determining concentrations of immunosuppressants across the entire clinical range with high sensitivity and robustness.

Sample preparation is usually simple and it is possible to detect several immunosuppressants simultaneously leading to better operational efficiency, shorter turnaround time and reduced cost per reportable result.

Principle and Method of Estimation of Immunosuppressants by LC-MS/MS:

The analysis is commonly performed using electrospray positive ionization (ESI) in the multiple reaction monitoring (MRM) mode.

Multilevel calibrator set of known concentrations, isotopically labelled internal standards, internal quality control (IQC) at clinically relevant low and high concentrations and participation in appropriate external quality

assurance program (EQAS) must be used as per individual laboratory's quality control policy to ensure accuracy of reports.

Many well-established home brew methods are available for estimation of Immunosuppressants by LC-MS/MS apart from commercial, IVD reagent kits (CE/US FDA approved) from reputed manufacturers. Laboratories may choose to validate their own inhouse methods or use commercial, reagent kits following method verification as per individual requirement. Laboratories must ensure that, only LC-MS/MS grade reagents and water is used for analysis.

Method verification by laboratories must include intraassay and interassay precision check, determining limit of quantification (LOQ) and limit of detection (LOD), evaluation of matrix effect and accuracy check by analyzing proficiency testing (PT) samples (if available) or interlaboratory comparison (ILC) with a CLIA accredited laboratory using LC-MS/MS based method for estimating immunosuppressants.

Specimen Requirement and Patient Preparation:

Whole blood sample (EDTA or Heparin) needs to be sent [4]. Cold chain (2-8 degree Celsius) must be maintained from collection to analysis.

Mentioning the time of sample collection and administration of the particular immunosuppressant drug (trough level or peak level) in test requisition form is mandatory as therapeutic ranges or targets are based on samples drawn at trough (immediately before a scheduled dose) or Peak (usually 2 hours after a dose) phases. Blood drawn at other times will yield higher or lower results [5].

Conclusion:

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is considered the "Gold standard" method or method of choice for quantitative measurement of immunosuppressants as it is free from the limitations inherent to immunoassays. The high selectivity of this method prevents the overestimation of drug concentrations in patient samples. However, the principal advantages of LC-MS/MS can only be obtained when the integrity of sample is preserved, the instrument provides sufficient sensitivity, the method is traceable to the NIST standard and a proper validation of the analytical system has been carried out by the clinical laboratory. Only well-designed methods that are continuously controlled by internal and external quality control and supervised by trained manpower allow an accurate and stable measurement immunosuppressants, free of any analytical error.

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