liquid chromatographic (HPLC) method with UV detection at 330 nm was developed for detection of dialyzed Erlotinib in protein-binding studies in the presence of albumin. Equilibrium dialysis method with fast spin dialyzer and 25 KD Nitrocellulose filters were used. A reversed-phase Symmetry C18 column (250 mm x 4.6 mm, 5 µm) was used at room temperature. The mobile phase was a mixture of methanol, acetonitril and potassium dihydrogen. Analysis was run at a flow rate of 1.3 ml/min. The run time for Erlotinib was approximately 7 minutes. The method was validated for its specificity, linearity, accuracy and precision.Therefore a simple, accurate and precise reversed-phase isocratic HPLC method with UV detection has been optimized and validated for the determination of erlotinib in human plasma.

Keywords: Erlotinib, Protein-binding, HPLC, Plasma.

Abstract No.237

Interaction of Erlotinib Hydrochloride with Human Serum Albumin

*Arash Khodaei**, *Leila Hassani*, *Akram Hamidi*, *Elham Safar gholizadeh*, *Sevda Yousefian*

Department of Biological sciences, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, IR
(E-mail: arash.khodaei@iasbs.ac.ir)

Erlotinib hydrochloride is a drug used to treat non-small cell lung cancer (NSCLC), pancreatic cancer and several other types of cancer. Erlotinib specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. The human serum albumin (HSA) is a major plasma protein. It is named a multifunctional plasma carrier protein because of its ability to bind to an unusually broad spectrum of ligands. HSA binds to the number of drugs altering their pharmacokinetics. Thus, the interaction between erlotinib hydrochloride as a drug with human serum albumin is significant. In this study, experimental investigation on the interaction of erlotinib hydrochloride with human serum albumin was carried out.Stern–Volmer dynamic quenching constant, binding constant and the number of binding sites for interaction of erlotinib hydrochloride with HSA were measured using analyzing of the fluorescence spectroscopic data. The intrinsic fluorescence spectra indicated that the intensity of fluorescence emission decreases as a function of erotinib concentration indicating the partial opening of the protein structure upon interaction with the drug. Fluorescence measurements on HSA–ANS complex was carried out to give information on the variations of HAS accessible hydrophobic areas and compactness of the protein. The increase in ANS emission indicated that the drug affects on the hydrophobic accessible surface area of HAS and leads to exposing of its hydrophobic groups.

Keywords: Erlotinib, HSA, Interaction, Spectroscopy.

Abstract No.238

Rolling Circle Amplification Technique in Medical Diagnosis

*Vahid Hamidi**, *Hedayatollah Ghourchian*

PTEN, Tumor Suppressor Gene, Gene Mutation, Gastric Cancer, IR
(E-mail: vhamidi@ibb.ut.ac.ir)

Due to its robustness and simplicity, the Rolling-Circle Amplification (RCA) of circular DNA probes holds a distinct position in DNA based diagnostics among other isothermal detection methods. RCA reactions exhibit an excellent sequence specificity that is favorable for genotyping or mutation detection, antigen detection, DNA based biosensors and allows to unambiguously identification of DNA markers on the excessive unrelated background. We use circular DNA as a template for RCA reaction. When primers anneal to the template in presence of nucleotides, reaction buffer and phi29 DNA polymerase, RCA proceeds and make long strand DNA. In the presence of two primers, a complex pattern of DNA strand displacement ensues that generates 109 or more copies of each circle in 90 minutes. Using a single primer, RCA generates hundreds of tandem linked copies of a covalently closed circle in a few minutes. An important factor for the success of this method is the unique nature of phi29 DNA polymerase which has excellent strand displacement activity. The use of primers with 3´ thiophosphate-protected ends is also important, allowing circular DNA molecules to be amplified at least 10,000-fold by protecting the primers from the 3´_exonuclease activity of phi29 DNA polymerase. To achieve amplification, phi29 DNA polymerase appears to initiate multiple replication forks on each circle and to perform exponentially cascading strand displacement amplification. These results indicate that circular DNA probes can be amplified to the high levels required for solution based DNA diagnostics.

Keywords: Rolling Circle Amplification, Phi29 DNA polymerase, Displacement Activity, Isothermal Amplification.
