In order to investigate the composition of skin or the drug penetration of topically applied substances into the skin, several well known analytical methods exist. They are mainly based on taking samples from the tissue and following analysis. One of the most used practices is the excision of the stratum corneum layers by means of adhesive strips. The adherent epidermal layers afford the stratum corneum to be analysed depth resolved. The so called tape stripping is a minimally invasive procedure and has become a standard method in many in vivo studies. Dermal microdialysis, tape stripping or extractions by organic solvents are often called relatively non invasive techniques. However, genuine non invasive methods can give much benefit in study design and especially under in vivo conditions. Fourier-transform infrared photoacoustic spectroscopy (FTIR - PAS), photothermal beam deflection (PBD) and Raman spectroscopy belong to the modern innovative non invasive analytical tools that are beginning to be recognized as high potential techniques for the non invasive study of biological tissues and human skin under in vitro and in vivo conditions. They can be applied to obtain information regarding the molecular composition of the skin down to several hundred micrometers below the skin surface. All three methods allow depth resolved investigations. While PAS and PBD use a frequency modulation of the excitation beam to reach deeper regions in sample, the principle of a confocal Raman microscope (CRM) is the movement of the specimen in the focal plane. In consideration of depth measurements PAS and PBD complete the applicable spectrum of the CRM, since Raman microscopy requires particular transparent materials. The enormous advantage of spectroscopic techniques is that the drug content can be determined directly without or at least with minimal sample preparation. This simplification of the analytical procedure yields a significant increase of the analyses reproducibility. Furthermore, on line spectroscopic measurements permit to follow the kinetics of reactions and allow depth profiling. The objective of this task is to reveal the potential use of the (optical) spectroscopic methods, FTIR-PAS, PBD and Raman spectroscopy in pharmaceutical research and to show the potential of PBD and confocal Raman microscopy for 3 D mapping of the lateral diffusion of drugs into keratin membranes, stratum corneum and artificial membranes and as depth profiling techniques. Based on Fick’s second law the diffusion coefficient of the drug in the membrane was derived by numerical fitting of the experimental data. The numerical calculations were carried out using the non linear least square data fitting by Gauss Newton method.