SIMULTANEOUS METHOD FOR THE DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE BY HPLC: APPLICATION TO A PRE-CLINICAL PHARMACOKINETIC AND IN SITU PERMEABILITY STUDY.
Frashant B. Musmade$^1$, A. Karthik$^1$, A. Ranjith Kumar$^2$, Shriram M. Pathak$^1$, K. M. Bhat$^1$, S. Pandey$^2$ and N. Udupa$^2$.
$^1$Dept of pharmaceutical Quality Assurance, $^2$Dept of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal- 576104, Karnataka, India.

Abstract
Various HPLC-UV methods are reported in the literature for the estimation of pioglitazone (PIO) or glimepiride (GLE) alone in plasma. The present study focused on developing and validating HPLC-UV method for simultaneous estimation of PIO and GLE in the plasma (rat human) and also in the samples obtained from the permeability study done in rats. The extraction method involved a solid phase extraction (SPE) using Oasis HLB cartridge (1cc, 30mg). Ritonavir was used as internal standard. Separation of the analytes was achieved within 15 min using a reversed-phase (Grace Vydrac C18) analytical column (150 × 4.6 mm i.d., 5 μ particle size) with a mobile phase of acetonitrileammonium acetate buffer (10.0 mM with 0.1% TEA; pH 5.0 adjusted using glacial acetic acid) at a composition of 48:52 % v/v. The flow rate used was 1.0 mL/min. The eluent was monitored at a wavelength 230 nm. The developed HPLC method was validated as per US-FDA guideline and the results met the acceptance criteria as per the guideline. The method was proved to be accurate and precise at a linearity range of 50–8000 ng/mL for PIO and 50.0-2000.0ng/mL for GLE with a correlation coefficient (r2) of 0.990. The method was robust with a lower limit of quantitation of 50.0 ng/mL for PIO and GLE. This method was used for the estimation of both the drugs in rat plasma obtained from the pharmacokinetic study of the drugs alone and in combination in rats. The same method was also used for the estimation of PIO in the samples obtained from the intestinal permeability study of PIO. This study was performed to determine the effect of flavonoids such as quercetine on the intestinal absorption of PIO. Results indicated that there was significant increase in the permeability coefficient (Peff) of PIO in presence of quercetine.

POSSIBILITY OF AN EFICACIOUS ADMINISTRATION PLAN USING DRUG PROTEIN BINDING INHIBITION IN HEMODIALYSIS
Toyotaka Nishio$^{1,2}$, Norito Takamura$^3$, Jin Tokunaga$^3$, Kenji Ogata$^3$, Ryuichi Nishii$^4$, Mitsuyoshi Yoshimoto$^1$ and Keiichi Kawai$^{1,5}$
$^1$Kanazawa University Graduate School of Medical Science 5-11-80 Kodatsuno, Kanazawa, Ishikawa 920-0942, $^2$Novapharmacy, 1170-1 Shimowada, Yamato, Kanagawa 242-0015, $^3$School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, $^4$Research Institute, Shiga Medical Center, 5-4-30 Moriyama, Moriyama-City, Shiga 524-8524 and $^5$University of Fukui Biomedical Imaging Research Center, 23-3 Matsuokashimoaiizuki, EIheiji-cho, Yoshida, Fukui 910-1193, Japan

We have studied the efficacious administration plan using the inhibition that may occur between two drugs at the same site of human serum albumin (HSA). Therefore, to the study on the possibility of an administration plan using binding inhibition in hemodialysis (HD) patients with end-stage renal disease, we investigated whether the fluctuations would occur between pre- and post-HD. In the method, we evaluated free fractions of site-probes, $^{13}$C-warfarin (site I) and $^{13}$C-diazepam (site II), by ultrafiltration in serum between pre- and post-HD. Endogenous uremic toxins, 3-carboxy-4-methyl-5-propyl-2-furanpropionate, indoxyl sulfate and hippurate, were determined by HPLC. We referred to the clinical laboratory test results for the concentrations of HSA and free fatty acid (FFA). As a result, the free fractions of $^{13}$C-warfarin at site I remarkably decreased in all 14 patients at post-HD compared to pre-HD. The free fractions of $^{13}$C-diazepam at site II remarkably decreased in10 of 14, and unexpectedly increased in 4 (− increment of inhibitory effect). We suggested that the factors in the increments of the binding affinities of site I and II were hemococoncentration and the decrements in the uremic toxins concentrations by HD but in the decrement of the binding affinity in site II was the remarkable increments of the FFA concentrations in 4. Consequently, we suggested that the administration plan, using binding inhibition, is possible to utilize HD.