**Experiment 6**

Determination of gliclazide binding parameters to plasma proteins

**Theoretical knowledge**: drug binding, sites of drug binding, binding constant, fraction bound; Scatchard, Klotz, and Rosenthal equations; determination of free drug concentration; influence of drug binding on pharmacokinetics parameters

The aim of the experiment is to determine the fraction of gliclazide bound to plasma proteins using ultrafiltration method.

Experimental procedure:

**1. Preparation of a mixture containing human plasma and gliclazide**
Add 100 µl of gliclazide solution (40 *µg/mL)* to the tube containing 400 µl of human plasma and incubate it at $37^0C$ for 1 hour to equilibrate it.

**2. Determination of the calibration curve for gliclazide.**

- In plastic micro tube prepare solutions at following compositions:

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|---|
| 1. Plasma sample filtrate | 400 µL | 400 µL | 400 µL | 400 µL | 400 µL |
| 2. Gliclazide solution | 100 µL *(2.5 µg/mL)* | 100 µL *(5 µg/mL)* | 100 µL *(10 µg/mL)* | 100 µL *(20 µg/mL)* | 100 µL *(40 µg/mL)* |

- 100 µL of each solution transfer in to glass vial and inject onto the chromatographic system.

Chromatographic conditions : A chromatograph model HP 1100 (Hewlett-Packard, Waldbronn, Geramny) will be use for determination of gliclazide.
LiChrospher 100 C 18 (5 µm) 125 x 4 mm with a precolumn LiChroCart 4-4 packed with a LiChrospher C 18 (5 µm) sorbent (both Merck, Germany) will be use as analytical columns. The mobile phase: mixture of 0.04 M potassium dihydrogenphosphate (pH = 3.8) and acetonitrile (51 : 49; v/v). Flow rate: 1 ml/min and the gliclazide peaks detection: 226 nm.

- Calculate the slope (a), the y-intercept (b) and regression coefficient of the calibration curve by the least square method.

**3. The determination of the fraction of gliclazide bound to albumin**

The determination of the initial gliclazide concentration

- Add 100 µl of gliclazide solution (40 *µg/mL)* to the micro tube containing 400 µl of human plasma and vortex it.
- 100 µL of prepared solution transfer to glass vial and inject onto the chromatographic system.

The determination of the concentration of unbound (free) gliclazide.

- Aliquots of 500 µl of the reacting mixture after incubation transfer to Millipore tube.
- After centrifugation for 15 min at $37^0C$ and 16000 rpm take sample of 100 µL, transfer it in to glass vial and inject onto the chromatographic system.

Calculate the total and free gliclazide concentration using the calibration curve parameters.

Determine the fraction of gliclazide bound to albumin using the following formula:

$$\beta = \frac{C_t - C_f}{C_t} \quad \mathbf{100\%}$$

where:

$C_t$ – initial concentration of gliclazide

$C_f$ – concentration of free gliclazide

**4. Write the laboratory report using a general format, as follows:**

a. title
b. purpose
c. calculations
d. results
e. graphs
f. conclusions