Abstract

In the first part of this doctoral thesis, a new analytical HPLC-MS/MS method for monitoring of concentration changes of 17β-estradiol (βE2) during *in vitro* mouse sperm capacitation was developed. Capacitation was performed for three initial concentrations of βE2 (200, 20 and 2 μg/L). For all the concentrations a similar trend for the total unbound βE2 was observed. In general, the βE2 concentration decreased to reach its minimum and then increased again. The position of the minimum differed for the individual tested βE2 concentrations. Experimentally obtained results were subjected to the kinetic analysis. The curves fitted through the experimentally determined points displayed an autocatalytic character. For the agreement between the curves obtained by fitting through the experimental points and the theoretical calculated curves, it is necessary to assume that the first step is adsorption of βE2 onto the surface of the sperm controlled by Langmuir isotherm.

The kinetic study was also used to study the effects of fluorides and aluminium fluoride complexes on the capacitation of mouse sperm. The experimental points were in very good agreement with the shape of the theoretical curves and this fact verifies the mechanism of the mouse sperm capacitation kinetics.

In the second part of this work, two analytical methods for chiral separation of aclidinium bromide (AB) and tapentadol hydrochloride (TAP) were developed.

Aclidinium bromide acts as a muscarinic receptor antagonist to relieve the symptoms of chronic obstructive pulmonary disease. The separation of 0.4% (*S*)-AB in a synthetic laboratory sample of AB by capillary electrophoresis was achieved using 4.8% (*w/v*) sulphated γ-cyclodextrin in an acidic background electrolyte based on potassium dihydrogen phosphate (100 mM, pH = 3.0) in an uncoated fused silica capillary with extended capillary light path. The method was validated as a limit test.

Tapentadol hydrochloride is a centrally acting analgesic for the treatment of moderate to severe acute pain or chronic pain. The TAP molecule has four possible stereoisomers, but only the (*R,R*)-isomer is currently a clinically used form. Enantioseparation of TAP by HPLC with resolution greater than 2.5 for all of the enantiomers was achieved using a Chiralpak AD-H column with heptane/propane-2-ol/diethylamine mobile phase (980: 20: 1, *v/v/v*). The developed method was validated according to the requirements of the International Conference on Harmonization (ICH).