Development and optimization of the UPLC-UV method for determination of chemical purity and assay of selected genistein co-crystals.

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Genistein is a natural organic compound that exhibits biological activity. It has anticancer, antimetastatic and antiangiogenic properties and estrogenic activity. The pharmaceutical use of genistein has become the subject of many medical studies [1]. The use of genistein in the development of effective drugs, pharmaceutical preparations, and supplements is difficult due to its low solubility in aqueous environments and the associated low bioavailability [2]. Co-crystal formation is one of the physical methods of improving the solubility of a drug and increasing its bioavailability. The FDA defines a cocrystal as a crystalline material composed of two or more different molecules, usually an active substance and a coformers, in the same crystal lattice, that co-exist with a defined stoichiometry and interact nonionically [4, 5]. In addition to structural studies, it is also important to determine the quality of the obtained crystal forms, to determine the chemical purity, stability and assay of the cocrystals.

The aim of the study was to develop selective methods using the ultrafast liquid chromatography technique in a reversed phase system with UV-VIS detection (UPLC-UV-VIS) to determine the chemical purity of selected genistein cocrystals and assay of genistein at the step of co-crystal preparation and in the studies of solubility and permeability of novel cocrystals.

Due to differences in polarity and pKa values of the compounds studied, in particular genistein and the cofomers were selected the most optimal conditions for UPLC method to at the same time determinate of genistein and of coformers, for example: stationary phase and mobile phase with bufor with the appropriate pH. Elution was retardeted for a coformers and the value of the retention coefficient (k) of the coformers was increased to an acceptable value in the range of 0.5-10.

The developed UPLC-UV methods are characterized by sufficient selectivity of the analyzed compounds, resolution (Rs) sensitivity and acceptable symmetry of peaks (A_s) from the determined components of the co-crystal and meet the criteria in accordance with the guidelines of the ICH and the European Pharmacopoeia [6, 7]. The sensitivity of the method was determined at the level of the concentration ~ 20 ng/mL where s/n for LOD is greater than 3. The methods are linear in the concentration range of 2 μg/mL-20 μg/mL for the genistein.

On the developed UPLC-UV methods, the solubility of genistein in different pH ranges of solutions was studied and the medium in which the solubility of genistein is the highest was selected. At the selected pH, the solubility and permeability of genistein and selected genistein cocrystals were studied and its assay in the cocrystals was determined. Solubility analysis in different pH ranges of the solution indicates the organ in the digestive system where the solubility of genistein will be the greatest.

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