17. PARTICULARITIES OF VALIDATION HPLC METHOD DOSING

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Introduction: Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids.Validation of a method is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose.

Materials and methods: Advanced bibliographic study.

Results: Though many types of HPLC techniques are available; the most commonly submitted method, the reversed-phase HPLC with UV detection, is selected to illustrate the parameters for validation.A. Accuracy is the measure of how close the experimental value is to the true value. Accuracy studies for drug substance and drug product are recommended to be performed at 80-100 and 120% levels of label claim as stated in the Guideline for Submitting Samples and Analytical Data for Methods Validation. B. Limit of Detection and Limit of Quantitation specifications are submitted with the regulatory impurities method relating to release and stability of both drug substance and drug product. C. Linearity range of detectability that obeys Beer's Law is dependent on the compound analyzed and detector used. D. Precision is the measure, that expresses the closeness of data values between a series of measurements obtained under unchanged analytical conditions. E. Range is the interval between the upper and lower levels of analyte studied. F. Recovery is defined as the observed result obtained from an amount of the analyts compared to the expected result obtained from theoretical amount and expressed as a percentage. G. Robustness is defined by ICH as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. H. Sample Solution Stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method.I. Specificity/selectivity: the analyte should have no interference from other extraneous components and be well resolved from them. J. System Suitability Specifications and Tests are parameters that provide assistance in achieving this purpose.

These parameters will be used to validate the method of assay of cinnamic acid from Tolu Balm in tablets.

Conclusions: The variations due to the drug product manufacturing process, the laboratory sample preparation procedure and the performance instrument contribute to the accuracy of the data obtained from the analysis. Only with good reliable validated methods, data, generated for release, stability, and pharmacokinetics, can be trust-worthy.

Keywords: HPLC, accuracy, detection limit, linearity, precision,range, recovery, robustness, stability, specificity

18. EVALUATION OF ACUTE TOXICITY OF POLYPHENOLS AND POLYSACCHARIDES EXTRACTS FROM *CENTAUREA CYANUS* L.

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Introduction: *Centaurea cyanus L.* is one of the species of *Asteraceae* family. It is an annual plant, growing as a weed in the fields. It is also used as ornamental plant due to its intense blue flowers. Cornflower has a long history of herbal use. The officinal vegetal product is *Cyani flores.* Externally it is used as anti-inflammatory and astringent for eye ailments and skin cleansing.The dried flowers have antipruritic, antitussive, weakly diuretic, emmenagogue, ophtalmic, very mildy

purgative, and tonic properties. According to our investigations aerial parts of *C. Cyanus* L. are an incontestable source of many phenolic compounds and polysaccharides. Pharmacological studies pointed out strong anti-inflammatory, gastroprotective effect of the *Cyani herba* selective extracts.

Purpose and objectives of this study was to test the acute toxicity of polyphenols and polysaccharides extracts from *Cyani herbain* on mice.

Material and methods: The polyphenols extract from *Cyani herba*was obtained by direct extraction with 60% aqueous ethanol and the polysaccharides one – with distilled water at 90°C. The total polyphenols and polysaccharides contents were determined in both extracts. Acute toxicity was evaluated in 84 mice (42 male and 42 female), weighing 18-26 g. Each extract was dissolved in constant volume of NaCl 0.9% (0.4-1 ml by oral route using intragastric syringe, and 0.2-0.5 ml – intraperitoneally). Initially animals were given 50, 250 mg/kg body weight (b.w.) of the extracts respectively, to possibly establish the range of doses producing any toxic effect. The animals were observed continuously for 7 days for any gross change in behavioural, neurological, autonomic profiles and mortality in each group.vSubsequently 500, 2000, 4000 mg/kg b.w. of the extracts were administrated. Mice were sampled after mortality for histopathological analyses of the selected tissues. The statistical analyses were carried out using Kurber's and Prozorovschi's methods.

Results: The acute lethal study of *Cyani herba* extracts in mice shows that enteral LD_0 was 4000 mg/kg b.w. LD 25%, LD 50%, and LD 100% haven't been established. Using intraperitoneal route LD_0 for both extracts was 250 mg/kg b.w. Intraperitoneal LD 17% for polyphenols extract was determined at 500, 2000, and 4000 mg/kg b.w. Intraperitoneal LD 34% for polysaccharides extract was established at 2000 and 4000 mg/kg b.w.

Conclusion: The obtained LD_0 value classifies the studied plant extracts as slightly toxic. The results suggest that the polyphenols and polysaccharides extracts of the aerial parts of *Centaurea cyanus* L. is relatively safe toxicologically when administrated orally and intraperitoneally.

Keywords: acute toxicity, *Cyani herba,* extracts, mice

19. METHODS OF DOSING FLAVONOIDS IN MEDICAL PLANTS

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Introduction: Flavonoids represent a group of useful compounds of vegetal origin that are of great interest for physiotherapy and pharmacology. This is a class of phenolic substances which gives its color to many species of flowers and fruits. Frequently, these pigments can be found in such plants as glycosides, in which one or more of the hydroxy groups of phenols are combined with reducing glucose. Based on the analysis, the effects of flavonoids can be grouped around the following biochemical processes: antioxidant and anti-inflammatory effect, and an important influence for the function of the immune system against asthma and allergies; in general could change the inhibition functioning of enzymes, viruses and bacteria effect. These benefits argue the objective of the study: dosing flavonoids in different part of the plant throught spectrophotometric and chromatographic techniques.

Purpose and objectives: To assess the methods of dosing flavonoids from various vegetable products: underground part, aerial parts, flowers, leaves and seeds.

Materials and methods: Vegetable products containing flavonosids: Silybi fructus, Calendulae flores, Menthae piperitae herba; Agrimoniae herba, Simphyti radices. Reference Standards - quercetin, rutoside, luteolin, silibinin, hyperoside. The analyzes were performed at perchin Elmer spectrophotometer Lambda 25 UV-VIS and high pressure liquid chromatography Jasco reversed phase.

Results: Flavonosids extraction was performed with ethyl alcohol 70%. For spectrophotometric determinations, were obtained extracts from the different part of the plant and analyzed in relation to alcoholic solutions of reference standards: quercetin, rutoside, luteolin, silibinin, hyperoside. The results were recalculated after standard dosage of quvercetin at wavelength 375 nm. Following results were