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HPLC FOR CONTROL STABILITY OF QUERCETIN INJECTABLE DOSAGE FORM

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Quercetin is a flavone derivative which conventionally attributed to a substances group with vitamin activity [1]. Currently, markedly increased interest in natural flavonoids, due to their high antioxidant, antimutagenic and anticarcinogenic activity and many other types biological activity [2, 3].

Despite a rather wide biological activity spectrum of quercetin derivatives, the degree of this activity varies greatly depending on the derivative, the substituents in the flavone structure, type and dosage form ant method their application. Polyphenolic nature structure does not allow a high bioavailability of pure quercetin when administered orally. This is associated with a wide spectrum variety chemical reactions for the phenolic groups: from interaction with amino acid residues in proteins to reactions with amine heterocycles alkaloids, polysaccharides.

Currently known quercetin based drugs for injectable use with drip pure form - Corvitin - has a pronounced anti-ischemic, and anti-stroke anti infarction activity [4-6]. As a result of preclinical studies have established that Corvitin has sufficiently low levels all types toxicity, allergenic and has no irritating action on intravenous administration [15].

Every year the number of quercetin dosage forms increases. This is due to the low toxicity and the drug substance availability with the high pharmacological effect [7-9]. This interest is constrained by the limited number of publications with analysis methods, especially HPLC [10, 12, 14]. The complexity of modern formulations forcing researchers to improve the HPLC for pharmacopoeias analysis, to validate this method for qualitative and quantitative methods for quercetin derivatives determination [11, 13].

The aim of our research was to determine the stability over time prepared from concentrate quercetin solution form, as well as to determine the stability of the concentrate to the original autoclave sterilization conditions.

Materials and methods

The object of research were quercetin soluble formulation samples: Sample 1 - fresh diluted concentrate (0.9% sodium chloride), Sample 3 - the same diluted concentrate, but which was stored at room temperature for 14 days in the light; Sample 2 - similar to the first sample diluted concentrate, but which went before breeding autoclaving at 120 ^{0} C for 20 minutes.

The objects of the studies used industrial drug-substance quercetin (Sinkea manufactured (China)), the original pharmaceutical composition as the soluble form quercetin for injection and aerosol applications, glycerol (Sigma), Polysorbat 80 (Merk), ethanol 96 %.

For the HPLC - analysis was used chromatograph "Milichrom A-02" (SiChrom, Knauer) (Econova, Novosibirsk, Russia). Considering the published data and own research, were selected optimal conditions of quercetin separation in the experiment. Chromatography methodology were standardized and validated for many drugs and presented in the database DB-2003 in the Milichrom A-02 software.

Spectral ratio for quercetin are shown in Table 1.

Table 1. The spectral ratios for the quercetin from Databases BD2003 Milichrom A-02

№ p / p	Code in database	Name	Vr, mkl	S_a210, o.u.*µl ---------- mg/ml	Spectral relations (S_λ/S210)						
					220	230	240	250	260	280	300
1.	A0162	Quercetine	1568	393.69	0.595	0.472	0.421	0.584	0.596	0.226	0.233

Speed eluent (flow) - 100 µl/min; Column ProntoSil-120-5-C18 AQ; column temperature 40^{0}C; detection in the UV range - 210-300 nm; mobile phase gradient chromatography to: [4M $LiClO_4$ - 0,1M $HClO_4$]: H_2O = 1: 19 and acetonitrile (analytical grade, Merck); injected sample volume - 4 µl; separation conditions gradient (0 to 100% acetonitrile. Initial non-aqueous concentrate quercetin contains as auxiliaries and cosolvents glycerol, ethanol, and polysorbate-80.

Excipients were needed to prevent quercetin's precipitation after introduction its concentrate in an infusion solution (glucose solution or saline). Before introducing the sample into the chromatograph the concentrate is diluted with saline to a final concentration of 0.01 mg / ml (1 sample). The first sample was analyzed at once (Figure 1a), the third sample was autoclaved before dilution (Figure 1b, the sample 3) and a second sample after dilution was allowed to air and light for 14 days at room temperature (Figure 2, Sample 2).

Results and discussion

Quercetin were identified using information on its retention time and spectral relations in the UV region from the database DB-2003 spectrums [16]. For spectrum polysorbate- 80 stock polysorbate's solution was prepared on a bidistilled water at a concentration of 1 mg/ml and then introduced into the database DB-2003. Pure polysorbate HPLC chromatogram and its UV spectra are shown in Figure 3.

The results of research are below:

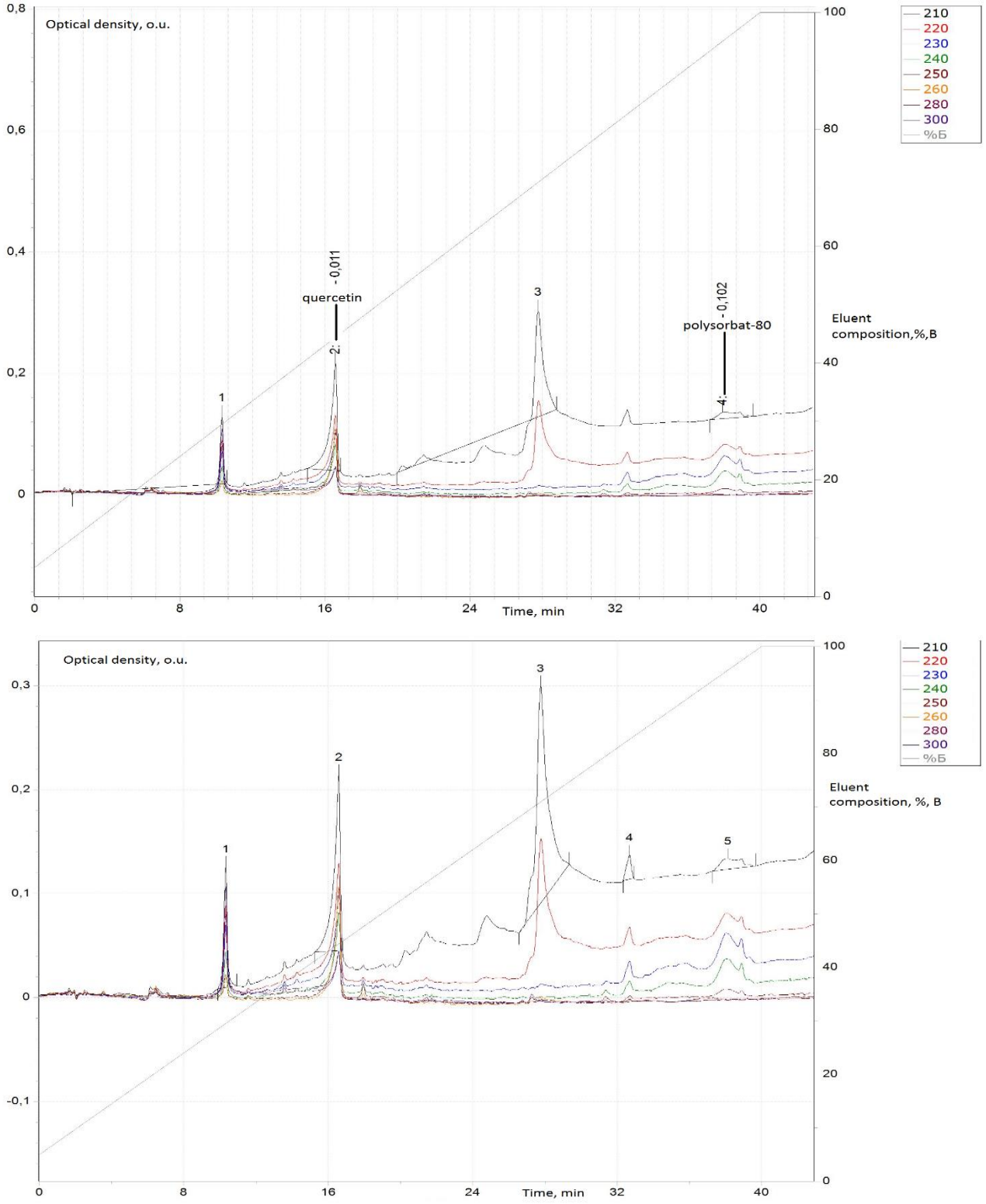

Figure 1. HPLC- chromatogram of freshly diluted quercetin concentrate (a) for infusion (sample 1): Peak 2 corresponds to the quercetin spectrum and peak 4 - corresponds to the polysorbat-80 range, chromatogram A and B (sample 3) are identical, indicating that the quercetin is stable in ampouled form under autoclaving and storage. Peaks 3 and 4 correspond to the polysorbat-80 background pollutants: 3- triphenylmethanol and 4-dibutylphthalat.

According to HPLC analysis results it can be established that in the process of separating compounds by chromatography column, characteristic peaks corresponding to the quercetin's in the database DB-2003.

A series of experiments was carried out under otherwise equal conditions after 14 days. Research objects were the same solutions prepared earlier. The results are displayed in Figure 2

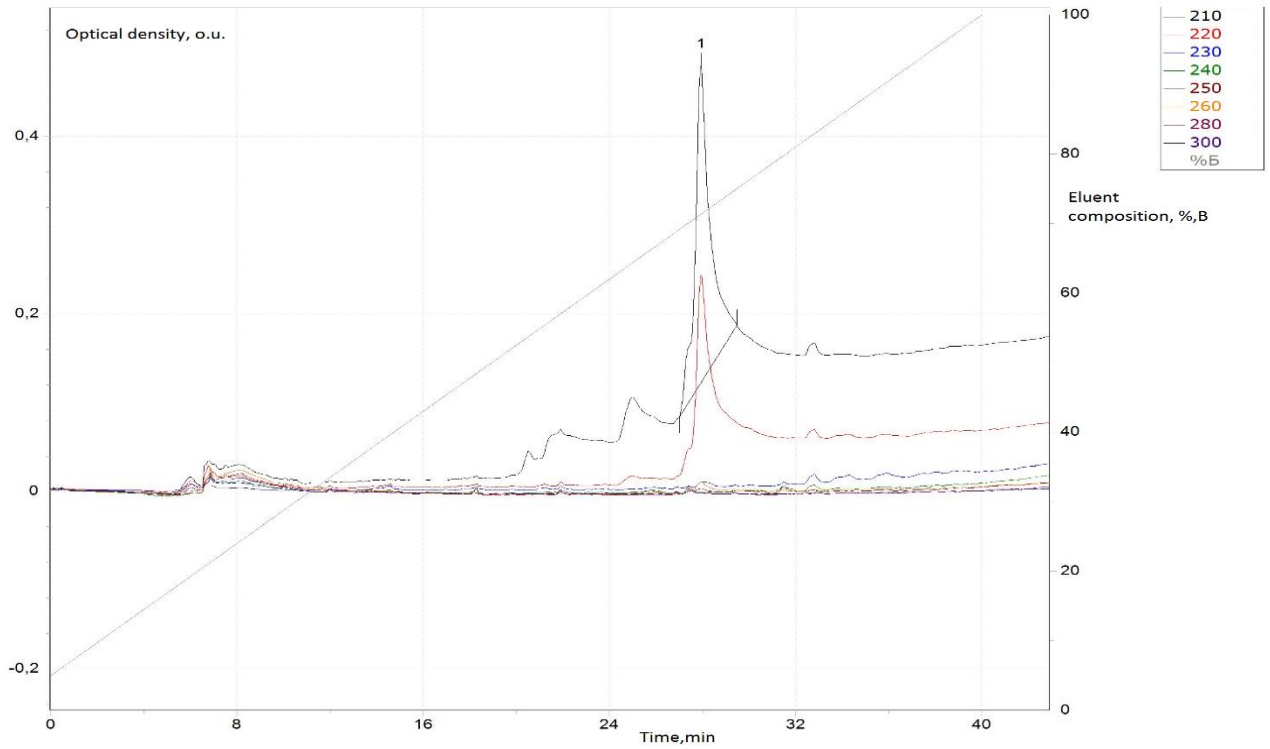

Figure 2. HPLC-chromatogram of the quercetin aqueous solution (sample 2), which was stored at room temperature for 14 days in the light: occurs quercetins complete degradation.

Figure 2 shows that the quercetins aqueous solution (sample 2) was subjected to the disintegration (absence of typical "peak" and the inability of chromatograph analytical system to identify quercetin and polysorbate-80 peaks).

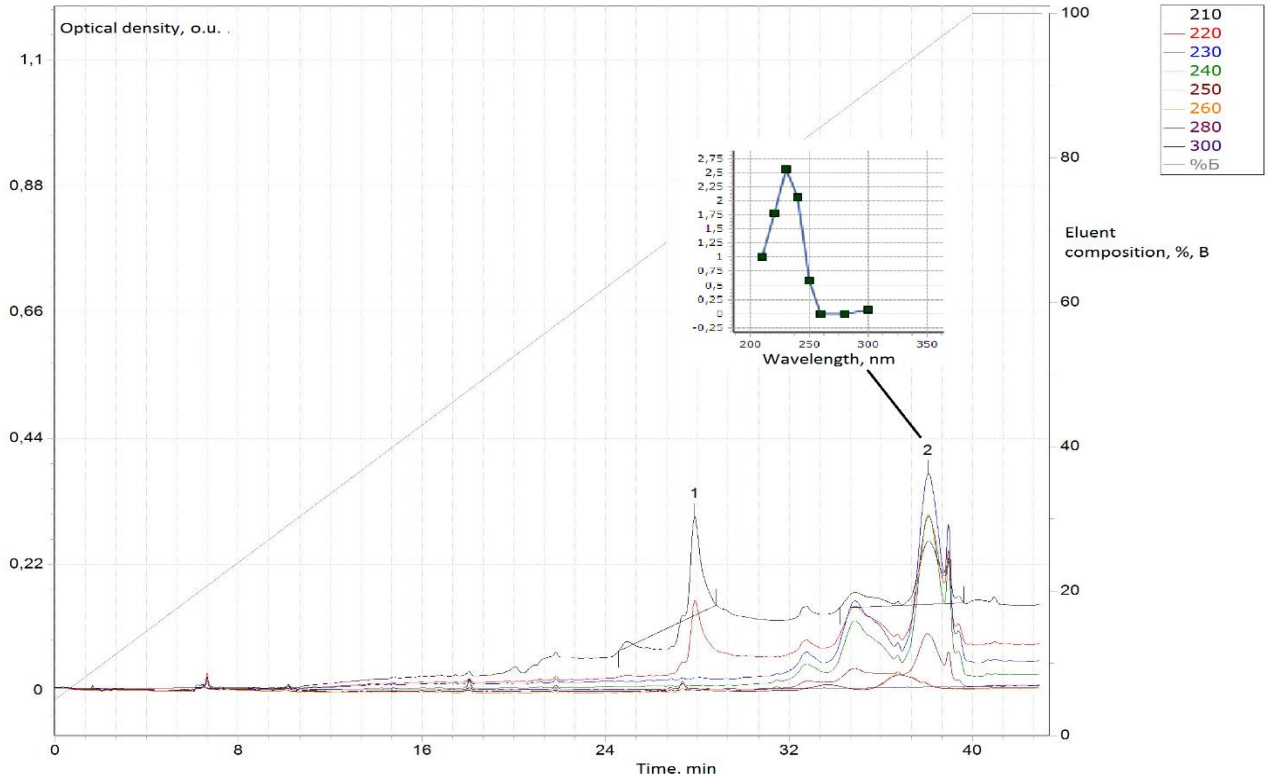

Figure 3. HPLC chromatogram of polysorbate-80 aqueous solution, the peak 2 is a major fully substituted sorbitol (Tween-80 or Polysorbate-80)

Also interesting earlier unknown fact that polysorbate-80 is full hydrolyzed to the initial compounds. Aqueous solutions of polysorbate-80 previously considered stable. The reason for the polysorbate-80 peaks disappearance can be as hydrolysis of ester groups and also mutual quercetin and polysorbate-80 oxidation reaction. This conclusion is based on the broadened peak appearance in the retention 6-8 min, that characteristic for the high hydrophilic compounds.

Conclusions

The analysis quercetin's perspective concentrates looking for extempore dilution were determined by HPLC chromatograph "Millichrome A - 02» (SiChrom, Knauer). It is shown that the HPLC methods using allows to establish the smallest difference in the samples.

The quercetin's non-aqueous concentrate is capable of withstanding retorting and standard indestructible in nonaqueous media (glycerol, ethanol, polysorbate 80)

Quercetin, although not precipitated when diluted with water, yet is unstable in aqueous solutions and are destroyed during prolonged storage, as well as an auxiliary solubilizer TWEEN-80. Need use aqueous solutions of quercetin for infusion only freshly prepared.

Gradient HPLC with UV- detection can be further used for quality control of quercetin and adjuvants injectable forms, and the method provides a sufficient reproducibility and enables to determine even solutions contaminants, such as dibutyl phthalate and triphenylmethane.

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Introduction. Quercetin is a flavone derivatives which known like a substances with vitamin activity, high antioxidant, antimutagenic and anticarcinogenic activity and many other types of biological activity. Wide usage of quercetin prevents their polyphenolic nature structure which does not allow a high bioavailability of pure quercetin when administered orally. This is associated with a wide spectrum variety of chemical reactions for the phenolic groups: from interaction with amino acid residues in proteins to reactions with amine heterocyclic alkaloids and polysaccharides. In our days Corvitin – one from the number of quercetin based drugs with sufficiently low levels all types toxicity, allergenic and has no irritating action on intravenous administration. In the same time quercetin cannot be used in full measure because of the limited number of publications with analysis methods, especially HPLC. Determining the stability over time of concentrate quercetin solution, as well as determining the stability of the concentrate to the original autoclave sterilization conditions is a promising direction in creating new drugs. **Materials and methods** The objective was to research quercetin soluble formulation samples in different conditions: 1) fresh dilute concentrate (0.9% sodium chloride); 2) the original dilute concentrate, which was stored at room temperature for 14 days in light and 3) similar to the first sample dilute concentrate, which went before breeding in autoclaving at 120 ^{0} C for 20 minutes. The objects used in the studies were industrial drug-substance quercetin (Sinkea

manufactured (China)), the original pharmaceutical composition as the soluble form of quercetin for injection and aerosol applications, glycerol (Sigma), Polysorbat 80 (Merk), ethanol 96 %. For the HPLC – analysis, chromatograph "Milichrom A-02" (SiChrom, Knauer) (Econova, Novosibirsk, Russia) was used. **Results and discussion** Quercetin was identified using information on its retention time and spectral relations in the UV region from the database of DB-2003 spectrums. HPLC analysis results show that the quercetin is stable in ampouled form under autoclave and storage and freshly diluted quercetin concentrate for infusion are identical. Quercetin aqueous solution which was stored at room temperature for 14 days in the light, turned out to be unstable. It was found that aqueous solutions of polysorbate-80 was full hydrolyzed to the initial compounds. **Conclusions** In this work the ability of quercetin's perspective concentrates to be stable were checked. The stability of concentrates was determined by HPLC chromatograph "Millichrome A - 02» (SiChrom, Knauer). It is shown that the HPLC methods can be used to establish the smallest difference in the samples. The quercetin's non-aqueous concentrate is capable of withstanding retorting and remains in standard indestructible state in nonaqueous media (glycerol, ethanol, polysorbate 80). Quercetin is unstable in aqueous solutions and are destroyed during prolonged storage. HPLC- chromatogram is presented in the article and show that gradient HPLC with UV- detection can be used for quality control of quercetin.

Key words: quercetin, stability, HPLC, autoclave sterilization, polysorbate-80.