# DETERMINATION OF CAFFEIC ACID IN HERBAL PREPARATIONS

Khanina Nataliia, Kanin Vadim, Georgiyants Victoriya
***The National University of Pharmacy, Kharkiv, Ukraine***
*natalykhanina@gmail.com*

Nowadays, the use of herbal preparations is gaining more and more popularity Especially the use of generic drugs. Possessing almost the same high efficiency as synthetic analogs, herbal preparations nevertheless have a milder effect on the body, eliminating severe side effects. Due to the rapid development of the Covid-19 pandemic, many herbal preparations have become the standard of care for the symptoms of the virus and the prevention of immunity.

One of several such drugs is "Sinupret" of the pharmaceutical company "Bionorica". During post-marketing research, our goal is to study the exact chemical composition and concentration of active substances.

For most herbal preparations, determining the exact chemical composition of the components is the most important task.

**Aim.** Thus, the purpose of this work is the gradual discovery of the composition of the drug and the establishment of the exact concentration of active substances. The object of the study is Caffeic acid, one of the many components that are part of the combined herbal preparation Sinupret.

**Materials and methods**. To study the Caffeic acid in the composition of Sinupret and other components, we needed highly selective analytical equipment. Since usually such components in herbal medicines are presented as a complex mixture of chemicals of related chemical classes compounds. Therefore, the Agilent 6530 Q-TOF mass spectrometer detector was applied as a detecting system. This detector was used with the liquid chromatograph of Agilent 1290. HPLC - analysis was conducted in the next terms: chromatographic column Poroshell 120 EC - C18 (Agilent) (100 x 2,1 mm), the particle size of 2,7 microns; mobile phase (MP) A is 0,1% solution of formic acid in water; MP B is 0,1% solution of formic acid in acetonitrile; the gradient elution program: MP B from 0 to 90% for 40 minutes; ionizing mode – ESI+; a flow rate is 0,5 ml/of mines; the temperature of the column - 40 ºC; injection volume - 5 µl. Scan-out in the range of the masses of 50 – 1500 a.e. with the subsequent analysis of samples in the mode of tandem mass-spectrometry allowed to identify plant components.

**Results.** It has already been proven that caffeic acid phenethyl ester can bind to the highly conserved Mpro protein. SARS-CoV-2. may have the potential to inhibit the functional activity of the SARS-CoV-2 protease and may have some therapeutic value in the treatment of the deadly COVID-19 virus [1].

This method uses gradient elution to reliably separate identified and placebo components. Another advantage of the new technique was the use of a chromatographic column with a particle size of 1.7 µm. This made it possible, along with an increase in sensitivity, to increase the efficiency of the separation of the peaks of the specified substances.

On the given chromatograms of the full ion current, we can observe the peak of the substance with a retention time equal to 0.51 minutes. Analysis of the mass spectrum

of the substance corresponding to this peak showed that the mass of the molecular ion is 179.14 m/z.

According to information on standard mass spectra contained in the US National Center for Biotechnology Information (NCBI) Chemical Compounds and Mixtures Database – PubChem, this peak can be asserted with 95% confidence that this peak belongs to a substance that corresponds to Caffeic acid when considering the formation of a molecular ion according to the scheme $M + H^+$[2].

A comparison of the mass spectra of the substance under investigation, obtained for the tested solution and the Caffeic acid standard solution, allows us to make the conclusion that the peak observed on the chromatograms with a retention time of 5.7 min belongs to Caffeic acid. Thus, under the conditions of the developed chromatographic technique, it is possible to obtain an effective peak of Caffeic acid suitable for its quantitative determination.

A peak with a retention time of 0.53 minutes and a molecular ion mass corresponding to Caffeic acid is observed on the chromatograms of the full ion current obtained for the tested solution. Quantitative determination of Caffeic acid was carried out according to the external standard method.

**Conclusions.** The HPLC-MS methodology was developed under conditions of gradient elution. Caffeic Acid and other components contained in the Sinupret drug have been identified. It is planned to continue the study of the composition of the drug, as well as the application of the developed method in the study of the composition of other drugs.

**References**

1. Vipul Kumar, Jaspreet Kaur Dhanjal, Sunil C. Kaul, Renu Wadhwa & Durai Sundar (2021) Withanone and caffeic acid phenethyl ester are predicted to interact with main protease (Mpro) of SARS-CoV-2 and inhibit its activity, Journal of Biomolecular Structure and Dynamics, 39:11, 3842-3854, DOI: 10.1080/07391102.2020.1772108

2. PubChem source: https://pubchem.ncbi.nlm.nih.gov/