



## HESPERIDIN NANOCRYSTALS - ISOLATION, PURIFICATION AND NANONIZATION.

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### Abstract

Brazil is one of the world's largest producers of oranges, however after processing, approximately 50% of the fruit is discarded. The orange peel have many bioflavanoids and one of them is the hesperidin. It has antioxidant, anti-inflammatory and anticarcinogenic properties. The research has the principal objective to purify the hesperidin, after the extraction from the peel, using classic chromatography, since co-extraction of other bioflavanoids, such as diosmin and naringin occurs at the same time; as well as, to obtain stable nanometer particles of hesperidin, with potential for cosmetological applications.

### Key words:

Hesperidin, column chromatography, nanoparticles.

### Introduction

One of the project targets is to obtain the high purity hesperidin, since it is co-extracted with other bioflavonoids (diosmin and naringin) with similar structures and molecular weight, using column liquid chromatography method<sup>1-3</sup>.

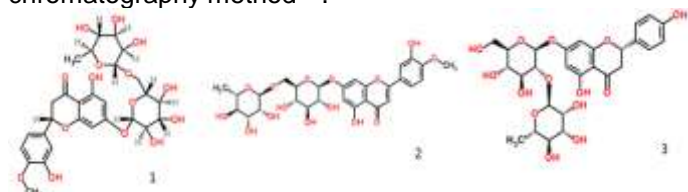


Image 1. Structures of hesperidin (1), diosmin (2) and naringin (3).

Another project target is obtaining stable hesperidin nanocrystals, in way the transdermal absorption is more efficient and the substance amount in the formulation can be decreased<sup>4,5</sup>.

### Results and Discussion

The hesperidin is extracted from the orange peel using the previously described method and that is frequently used<sup>1-3</sup>. The first objective of the project, which is the separation of the bioflavonoids (hesperidin, diosmin and naringin), was performed by thin layer chromatography using a reverse phase (C-18). Of the mobile phases tested, the one with the greatest capacity to separate the bioflavonoids was a phase composed from ethyl acetate and hexane. Diverse proportions of the mobile phase to optimize the separation were tested. Two mobile phases showed the best results, however the one that obtained the biggest difference between the values of R<sub>f</sub> is the mobile phase of ethyl acetate:hexane 16:3 (v/v). The R<sub>f</sub> obtained for hesperidin was 0.35, for diosmin was 0.24 and for naringin was 0.28. The fact that the difference in R<sub>f</sub> values does not represent many problems, because the main objective is obtain pure hesperidin.

The hesperidin nanonization was performed by applying the NANOEDGE™ technique, which consists of solubilizing the hesperidin, using a minimum possible of solvent, and mixing the solubilized hesperidin in a solvent where it is insoluble (water). Ultrasound to promote impact between particles was used. Two different formulations were prepared and their stability and estimated size of particles for the period of three months were monitored. The first formulation contained dimethyl sulfoxide (DMSO), glycerin, sodium carboxymethyl

cellulose and water. The second formulation contained orange oil, Pluronic F-127 and water.

Chart 1. Size and zeta potential of the particles of the hesperidin solution of the formulation 1 (0.05% of hesperidin)

Days	Size (nm)	Zeta Potential (mV)	Pdl
15	354.1	-51.7	0.297
33	320.1	-44.5	0.261
90	473.2	-26.2	0.314

Chart 2. Size and zeta potential of the particles of the hesperidin solution of the formulation 2 (0.10% of hesperidin)

Days	Size (nm)	Zeta Potential (mV)	Pdl
15	233.1	-22.1	0.461
33	236.2	-25.0	0.432
90	279.0	-21.1	0.459

### Conclusions

Mobile phase that achieved a satisfactory separation of the bioflavonoids in thin layer chromatography was selected, however it still should be tested in column. Stable hesperidin nanoparticles were obtained, although in formulation 1 there was greater variation in size and zeta potential over time. Formulation 2 maintained the values of size, zeta potential and polydispersity (Pdl) more constant, so it can be concluded that it presents greater stability.

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