

## OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF POLYMETHOXYLATED FLAVONOIDS IN *CITRUS SINENSIS*.

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*Citrus sinensis* is a fruit, commonly known as orange or sweet orange. It belongs to the family of the *Rutaceae*. *Citrus sinensis* is used in the traditional medicine in several countries. It is used for a.o. diabetes mellitus, relief of stomach pains, nausea, vomiting and gastrointestinal infections. The interest in its activity against many disorders is high, since *C. sinensis* has a wide range of compounds. One specific group of compounds is called the polymethoxylated flavonoids (PMF). PMFs appear to have a broad spectrum of activities, such as cardiovascular, anti-inflammatory and chemopreventive activities [1].

Many PMFs have already been identified, the two most common PMFs in *Citrus* are nobiletin and tangeretin. Since PMFs are often used for bio-activity studies, there was a need for an analytical method. We developed and optimized an analytical method for the quantification of the polymethoxylated flavonoids nobiletin and tangeretin in dried orange peel powder. In the final method nobiletin and tangeretin were extracted out of the dried orange peel powder using methanol 70% and the samples were analyzed on an HPLC-DAD-system. Validation of the method was done conform to the ICH guidelines on the validation of analytical methods [2]. A calibration model of both nobiletin and tangeretin standards were made in the concentration range from 1.8 µg/mL to 18.4 µg/mL and 0.4 µg/mL to 3.5 µg/mL respectively. The method was validated for the intermediate precision (3 days) and linearity (3 concentration levels). The recovery of the method was determined as 101.2% for nobiletin and 99.8% for tangeretin. For nobiletin, an average amount of 406.5 µg/g dried orange peel powder was found, with a RSD of 3.5%. For tangeretin, the average amount was 93.6 µg/g dried orange peel powder with a RSD of 0.96%.

[1] Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry* 2007; 104 (2): 466-479.

[2] ICH, Text on validation of analytical procedures – ICH Harmonised Tripartite Guideline, 1994.