Separation of quercetin from a Ginkgo Biloba mother tincture using the PrepChrom C-700

PrepChrom C-700: Pre-purification with flash chromatography followed by a prep-HPLC isolation of quercetin from the collected flash-fraction

Ginkgo Biloba has been used for curative reasons for centuries. The trees nuts have been an important element in the herbal practice of traditional Chinese medicine. Due to its beauty and its botanical importance Europeans started resettling and cultivating Ginkgo Biloba in Europe in the 17th century. In the second half of the 20th century scientist started taking interest in the medical properties of Ginkgo leave extract. [1] The positive effect on many illnesses, like age-related dementia and depression could be scientifically proven. Furthermore, the enhancement of memorizing and learning functions turned the attention to the Ginkgo Biloba leave extracts. [2]

1. Introduction

The aim of this application note was to isolate a flavonol, quercetin, from a Ginkgo Biloba mother tincture. The prepurification of the mother tincture was performed on a reversed phase flash column by the means of dry loading. The target compound was identified by its characteristic UV-VIS spectra and the retention time determined by running a standard. Subsequently, the purification of the pre-purified fraction of the target compound was performed with a 10 µm reversed phase prep-HPLC column.

2. Experimental

50 mL of the Ginkgo Biloba mother tincture was transferred into a 100 mL evaporation vessel and evaporated to dryness with the Rotavapor® R-300. The dry mass was determined on an analytical balance, subsequently, 10 times the amount of Silica RP-18 (LiChroprep® 25-50 µm) was added with 50 mL ethanol. This mixture was again evaporated to dryness. The silica remained free flowing and nothing stuck to the wall. 3.8 g of the prepared sample was transferred into the PrepElut adapter. The PrepElut adapter was tightened to avoid a dead volume.

Table 1: Chromatography conditions for the flash column (above) and prep-HPLC column (below)

Injection loop	Dry loading with PrepElut
Column	BUCHI Sepacore® C-18 25 g
Flow rate	20 mL/min
Detection	UV 220, 370, SCAN 200-600 nm
Solvent A	Acetonitrile / Water acidified with 1 % H_3PO_4
Gradient	Gradient: Acetonitrile (A) / Water 1 % H_3PO_4 (B) 10 % - 100 % Acetonitrile (A) in 20 min 100 % - 100 % Acetonitrile (A) in 3 min
Injection loop	10 mL
Column	BUCHI PrepChrom HPLC column C-18 10 µm, 150 x 21.2 mm
Flow rate	20 mL/min
Detection	UV 220, 370, SCAN 200-600 nm
Solvents	Acetonitrile / Water acidified with 1 % H_3PO_4
Gradient	Gradient: Acetonitrile (A) / Water 1 % H_3PO_4 (B) 10 % - 100 % Acetonitrile (A) in 20 min 100 % - 100 % Acetonitrile (A) in 3 min

The retention time of quercetin was determined by dissolving 50 mg of pure quercetin in 10 mL of 70 % ethanol. Subsequently, 2 mL of the solution was injected and run on both flash and prep-HPLC columns with the same gradient used for the separations.

3. Results

The pre-purification shows a reasonable separation efficiency, even at the very high loadings. Clearly, no volume overloading effects take an influence on the shape of the peak or the retention time. The retention time of the previously made up and separated quercetin standard was about 11 min. Accordingly the peak of interest could easily be identified. In addition, the quercetin could also be distinguished by its distinctive UV/VIS sprectrum provided by the scan function. One fraction was collected for the isolation of the compound of interest. Due to the better separation efficiency, a prep-HPLC column was employed for the purification of the flash chromatography fraction containing the quercetin. The smaller and unitary particle size of the modified silica particles in the prep-HPLC column can achieve a greater separation and purification performance. The chromatogram in Figure 1 shows the increase in the absorption signal of the wavelengths 370 nm (black) and 220 nm (gray) after 9 minutes. This was expected due to the similarity of the quercetin standard run for the chosen column. It is clearly shown that the separation could eliminate a second unidentified compound not visible at 370 nm, but at 220 nm that was also collected in the pre-purification step.

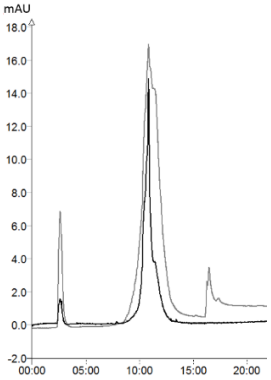

Figure 1: Recorded UV/VIS signals at the wavelengths 220 nm (gray) and 370 nm (black).

4. Conclusion

Quercetin can easily be separated from a crude mixture by first pre-purifying the mother tincture on a flash column and a HPLC purifying step to achieve the highest possible purity. Identification of this compound could be achieved with the scan function of the UV/VIS detector. The system is able to handle five UV/VIS signal inputs, thus an ideal method with four fixed wavelengths and one 200-600 nm scan can be generated for every separation. The prep-HPLC gives a significantly better separation of the components, due to higher number of separation plates. The PrepChrom C-700 can successfully separate complex natural compounds extracted from the Ginkgo Biloba leave.

5. References

[1] Van Beek, T.A. et al. (2005). *Ginkgo Biloba: Medicinal and Aromatic Plants – Industrial Profiles.* Volume 12. Amsterdam: Harwood Academic Publishers.

[2] Stargrove, M.B. (2008). *Herb, Nutrient, and Drug Interactions: Clinical Implications and Therapeutic Strategies.* St. Louis: Mosby Elsevier.

For more detailed information and safety conside-rations please refer to the Application Note No. 216/2015.