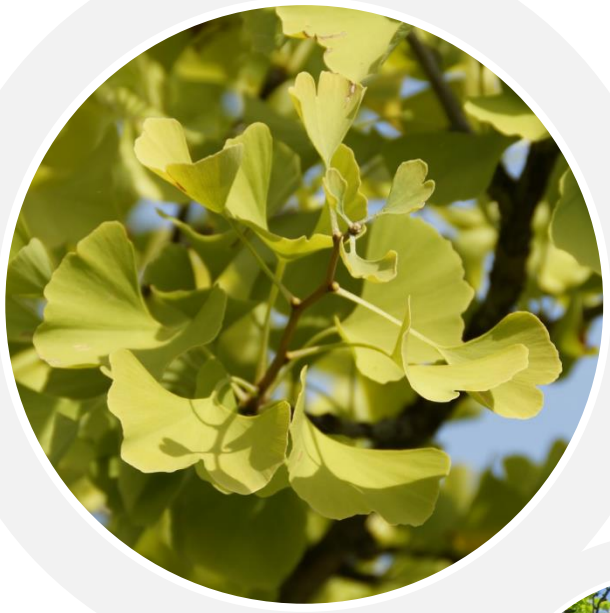
Application Note No. 216/2015

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



1. Introduction

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]
The aim of this application note was to isolate a flavonol, quercetin, from a Ginkgo Biloba mother tincture. The pre-purification 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.
BUCHI PrepChrom C-700 is capable of separating complex natural extracts. The UV/VIS-detector allows registering up to four different wavelengths and has a scan function to continuously record absorption spectra in the range of 200-600 nm.
Here we present an application to separate natural compounds by revesed phase flash chromatography as a pre-purification. Furthermore, the subsequent purification with a preparative HPLC column is also shown. A dry loading technique was used to demonstrate the high sample loading capacity of the system without broadening and shifting the peaks or have any interference with other solvents.

2. Equipment

- PrepChrom C-700
- Sepacore® C-18 25 g
- PrepChrom HPLC column C-18 10 µm, 150 x 21.2 mm
- PrepElut adapter
- Rotavapor® R-300
- Mettler Toledo Analytical balance

3. Chemicals and Materials

Chemicals:

- Acetonitrile (Brenntag-Schweizerhall AG, 81631-156, distilled)
- Distilled water (Brenntag-Schweizerhall AG, 11000-356, chemically pure)
- Ethanol (Brenntag-Schweizerhall AG, 64-17-5, 96 %, distilled)
- Phosporic acid solution (Sigma-Aldrich, W290017, 85 %)
- Ginkgo Bilobae mother tincture P44 (Hänseler, 25-7586-02, UN-Nr. 1293)
- Quercetin (Sigma-Aldrich, Q4951, ≥95% (HPLC))
- LiChroprep® RP-18 (Merck, 1.09303.0100, 25-40 µm)

Potential Health Effects: Consult respective material safety data sheets.

Protection: Wear appropriate protective equipment.

4. Experimental

4.1. Sample preparation

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.

4.2. Quercetin

Quercetin is a polyphenolic compound, similar to other flavonoids.It does not only appear in the Ginkgo Biloba leaves, it can also be found in many fruits and plants, such as berries, apples and onions. Quercetin acts as an antioxidant and can trap free radicals which can cause cell damage that eventually leads to cancer. Further beneficial properties concering this compound have been reported, such as anti-inflammoratory properties and the improvement of cardiovascular health. [3] In its pure state, the solid flavonol has a brown-yellow color. Quercetins absorption spectra shows strong absorbance in the near UV range.The UV/VIS absorbtion maxima of quercetin lies at 372 nm and 256 nm (Figure 1). The signals correspond to the chromophores consisting of the aromatic ring conjugated with carbonyl group and another aromatic ring containing the two hydroxide groups in meta and para position. The observed spectrum measured by the PrepChrom C-700 is consistent with the maxima found in scientific literature. [4]
The retention time of a quercetin was determined by dissolving 50 mg of pure quercetin in 10 mL of 70 % ethanol. Subsequently, 2 mL of the standard was injected and run on both flash and prep-HPLC column with the same gradient used for the separations.

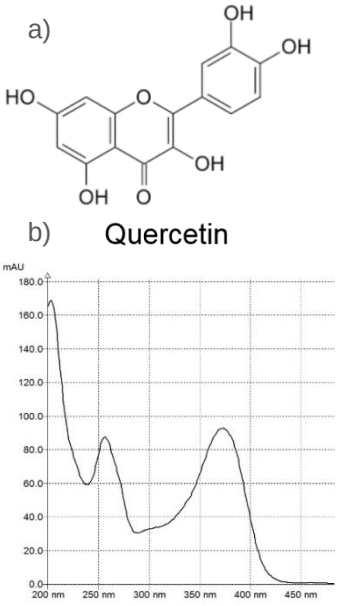


Figure 1. a) Quercetins molecular structure with b) the corresponding UV/VIS absorption spectrum.

4.3. PrepElut

The PrepElut adapter is used when loading dry, barely soluble or diluted samples. Dry loading involves dissolving the sample in a strong solvent followed by adsorbing the sample onto a stationary phase (such as silica) by removing the solvent in a Rotavapor®. The loaded silica can be transferred into a PP-cartridge and compressed from either side with a stamp to obtain the smallest possible dead volume.

Figure 2. PrepElut adapter

4.4. PrepChrom C-700 settings for the flash column

Table 1. Chromatography conditions for the flash column.

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
Solvent B	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

4.5. PrepChrom C-700 settings for the prep-HPLC column

Table 2. Chromatography conditions for the HPLC column.

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
Solvent A	Acetonitrile
Solvent B	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

5. Results and Discussion

5.1. Pre-purification of the Ginkgo Biloba mother tincture with the flash column

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, highlighted in green in Figure 3. According to this information the peak of interest could easily be identified. In addition, the quercetin could also be distinguished by its distinctive UV/VIS sprectra provided by the scan function. One fraction was collected for the isolation of the compound of interest.

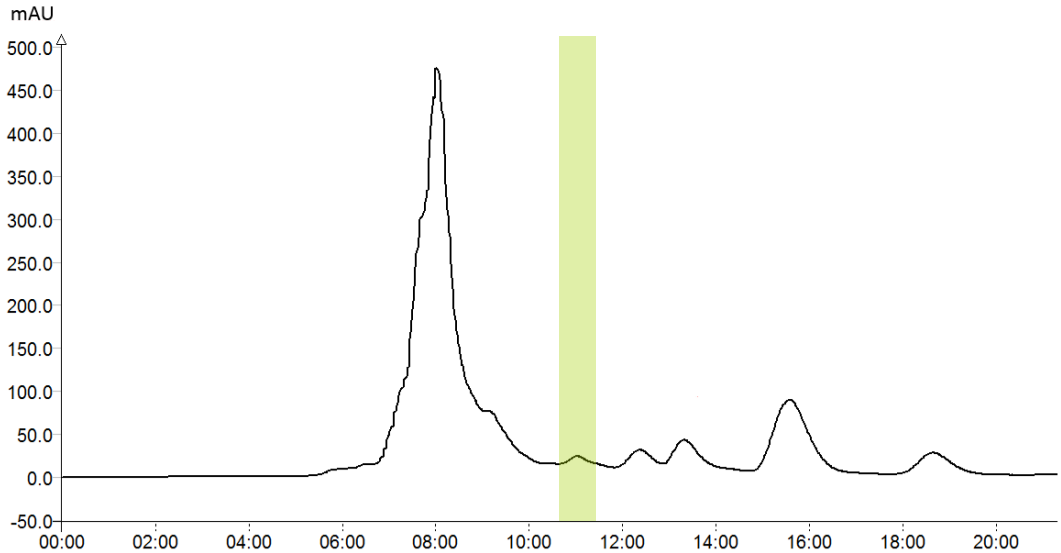


Figure 3. Recorded UV/VIS signal at the wavelength of 370 nm, one of quercetins distinctive UV/VIS maxima.

5.2. Isolation of the collected fraction with the prep-HPLC column

Due to the better separation efficiency, a prep-HPLC column was employed for the purification of the flash chromatography fraction containing the quercetin. The retention time and the flow rates where similar for both the flash and prep-HPLC separation. The pure quercetin peak is highlighted green in Figure 4 and was identified by a pure compound in a separate run. The smaller and unitary particle size of the modified silica particles in the prep-HPLC column can achieve a much greater separation and purification performance. The chromatogram, in Figure 4, shows the quercetin 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.

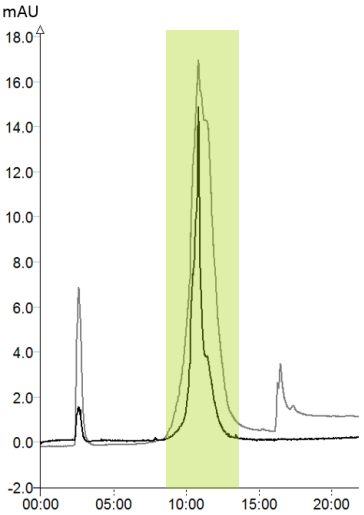


Figure 4. Recorded UV/VIS signals at the wavelengths 220 nm (gray) and 370 nm (black)**.**

6. 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.

7. 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.

[3] Global Healing Center (2013). *6 Health Benefits of Quercetin* http://www.globalhealingcenter.com/natural-health/health-benefits-of-quercetin/.

[4] Bondžić, A.M. (2013). *Spectrophotometric and Chromatographic Study of Reaction Between [Aucl4]- and Quercetin*. Belgrade: University of Belgrade.