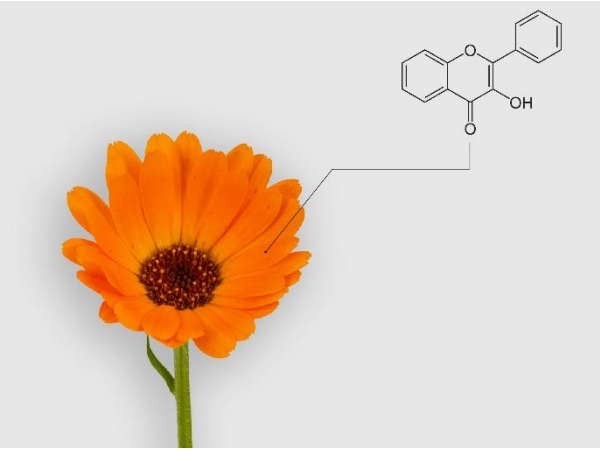
Determination of flavonoid content in *calendula officinalis* (marigold) *UniversalExtractor E-800: Extraction of marigold for the flavonoid determination*



1. Introduction

Since flavonoids are active substances in many plant materials, the determination of their content is of great importance, also for quality control reasons. In the presented application, the sample is extracted with the UniversalExtractor E-800 using the method Soxhlet warm. The determination of the flavonoids content is performed by using UV/Vis-spectrophotometry.

2. Experimental

Equipment: UniversalExtractor E-800

Powder from dried *calendula officinalis* marigold including the calyx, reference flavonoid content: 0.29%

Determination: The sample was weighed into a cellulose thimble. Hexamethylenetetramine-solution and hydrochloric acid are added. The samples were extracted using the UniversalExtractor E-800 (Figure 1) applying the parameters specified in Table 1. The sample was extracted in triplicate.

Table 1: Parameters for Soxhlet warm with the UniversalExtractor E-800

Extraction method	Soxhlet warm
Solvent	Acetone
Extraction Heating level	10 cycles 11 (Extraction) / 3 (Chamber)
Rinse step	5 min
Drying 1 Heating level	☑ AP 2 min 11
Solvent volume	100 mL

The extract is then transferred into a volumetric flask and filled up to 100 mL. A liquid-liquid extraction with water and ethylacetate is performed with a portion of the extract. The organic phase is collected, dried and filled up to volume, forming the stock solution. An aluminium chloride solution is added to a portion of the stock solution and diluted with glacial acetic acid in methanol. After 30 min, the absorbance of the test solution is determined and compared to a compensation liquid at 425 nm.

3. Results

The determined flavonoids content is presented in Table 2. The results are higher than the reference value determined with the manual extraction method specified in Ph. Eur. 07/09:3000, which was 0.29%. The deviation can be explained by the more efficient extraction and the avoidance of losses due to filtration and rinsing steps. The relative standard deviation lays within the intern tolerance of 5%.

Table 2: Determined flavonoids content of marigolds (rsd: relative standard deviation), n=3

Sample	Fat content	Mean value	rsd
Marigold	0.38% 0.36% 0.35%	**0.36%**	**4.18%**

4. Conclusion

The determination of flavonoids content in marigold powder by use of the UniversalExtractor E-800 provides reliable and repeatable results. Compared to the method described in Ph. Eur., the laborious filtration steps during the extraction, including the rinsing of the cotton wool, are omitted. The automation of the flavonoids extraction is thus a complete success.

5. Acknowledgements

We gratefully thank Mrs. Katharina Waldbauer from PADMA AG for the fruitful cooperation and for sharing her expertise and data for this application note. PADMA AG is a swiss pharmaceutical company, producing herbal medicines and food supplements based on Tibetan medicine.

6. References

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[2] Chen A., Xiang W., Liu D., Liu C., Yang L., Determination of Total Flavonoids and Its Antioxidant Ability in Houttuynia cordata, Complementary medicine researchJournal of Materials Science and Chemical Engineering, 4, 131-136, 2016.

[3] Williams R. J., Spencer J. P, Rice-Evans C., Flavonoids: antioxidants or signaling molecules?, Free Radical Biology and Medicine, 36, 838-849, 2004.

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For more detailed information and safety considerations please refer to the Application Note No. 417/2020.