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Application News

No. SCA_190_047

High Performance Liquid Chromatography

Analysis of Capsaicinoids in Chili Products

▪ Introduction

Pungency of chili products depends on the amount of capsaicinoids which are naturally present in bell peppers or chili peppers. The two main components, capsaicin (69%) and dihydro-capsaicin (22%), are almost twice as strong as the capsaicinoids nordihydrocapsaicin (7%), homodi-hydrocapsaicin (1%) and homocapsaicin (1%), which are smaller in comparison [1]. Therefore, only capsaicin and dihydrocapsaicin were studied to determine capsaicin levels in chili products.

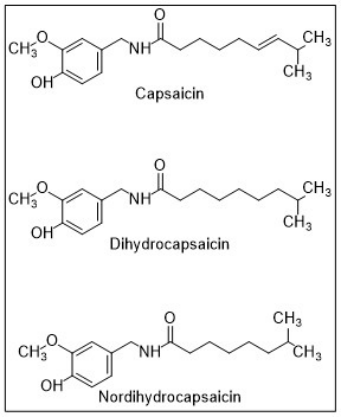

Fig.1 Chemical Structures of Capsaicinoids

The pungency level of a pepper is measured in Scoville Units, defined by Wilbur Scoville. Scoville used a group of tasters to determine the dilution needed to remove the pungency from a sauce or food [2]. Pure capsaicin is rated at 16,000,000 Scoville units.

▪ Method

An isocratic system equipped with a photodiode array detector (PDA) and fluorescence detector was used for the analysis. The method parameters are shown in table 1.

Table 1: Analytical Conditions

System	Nexera X2 + SPD-M30A + RF-20Axs
Column	Shim-Pack GIST C18 2 µm; 2.1 x 100 mm
Mobil Phase	A: 1 % Acetic Acid in H_2O; B: Acetonitrile
Method	A: 60 Vol.-% B: 40 Vol.- %
Flow Rate	0.9 ml/min
Detection	PDA 280 nm RF-20Axs: Ex 280 nm, Em 325 nm
Temp.	50 °C
Inj. Vol.	1 µl
Run Time	6 min

Fluorescence detection exhibits approximately 16-times higher sensitivity than detection with the PDA. Therefore, the focus of this study was only on the fluorescence detector for further method development.

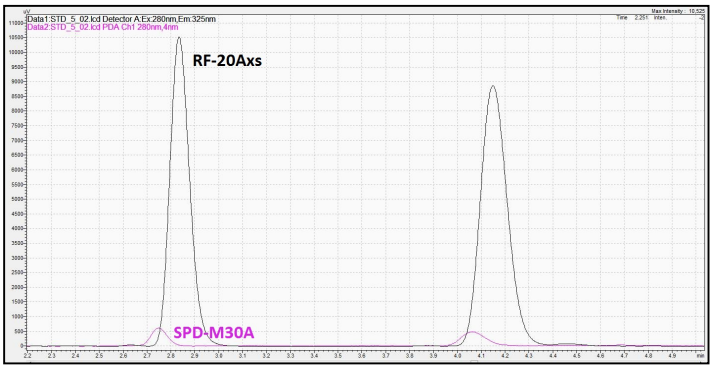

Fig. 2: Comparison PDA vs. Fluorescence Detector

▪ Calibration

The two capsaicinoids capsaicin and dihydrocapsaicin were analyzed. Figure 3 shows a chromatogram of a capsaicinoids standard solution with a concentration of 5 µg/ml of each analyte.

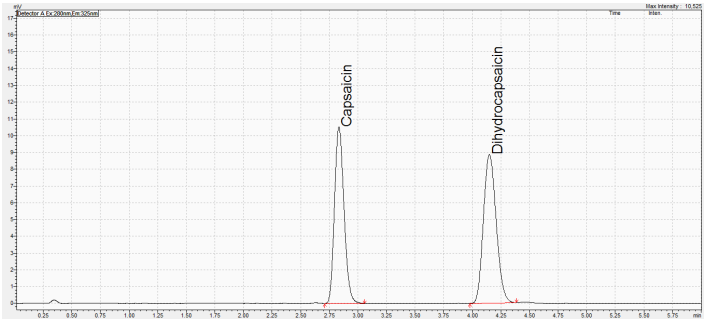

Fig. 3: Chromatogram of a Standard (5 µg/ml)

In order to enable quantification of the capsaicin content, six point calibration curves in a concentration range of 5 – 100 µg/mL were created for each analyte. Calibration curves and the corresponding coefficient of determination R^2 are shown in figure 4.

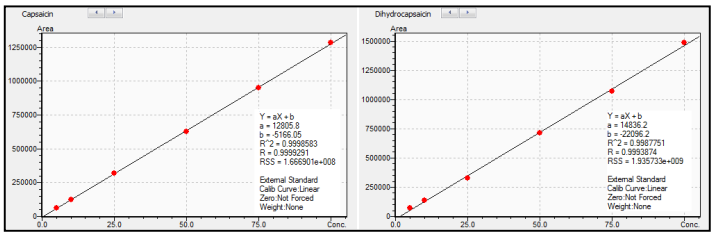

Figure 4: Calibration Curves of the two Capsaicinoids

▪ Sample Preparation and Measurement

Figure 5 shows the sample preparation for the chili products.

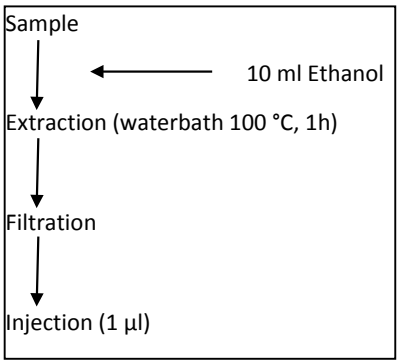

Fig. 5: Sample Preparation

An example of a chromatogram of a chili sauce is shown in figure 6. According to literature [3] it is assumed based on the elution order that the peak at 2.6 min is nordihydrocapsaicin.

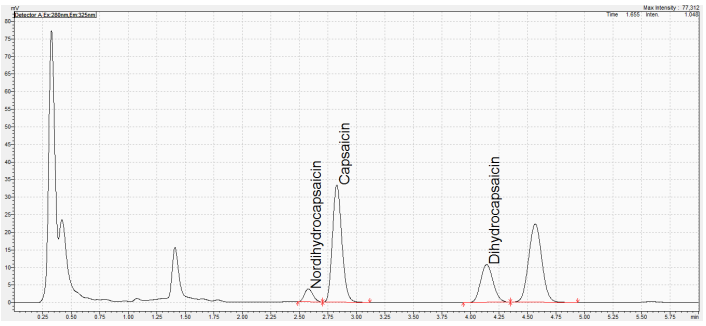

Fig. 6: Chromatogram of a Chili Sauce

▪ Summary

This application note describes the analysis of the two main capsaicinoids of chili peppers using a fluorescence detector in combination with a Nexera X2 UHPLC system. All capsaicinoids elute within 4.5 minutes and were baseline separated. The method was calibrated in a concentration range of 5 to 100 µg/ml and linearity in this range was shown by a coefficient of determination R^2 of ≥ 0.998 for all analytes.

[1] Bennett DJ, Kirby GW (1968). J. Chem. Soc. C: 442
[2] Collins MD, Wasmund LM, Bosland PW (1995 HortScience. 30 (1): 137–139
[3] AOAC Official Method 995.03

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