Pungency level determination of chili products with HPLC

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1. Introduction

Every culture has its own famous street food. The UK is famous for its fish and chips, Denmark for its hot dogs, The Netherlands for Frikandel, and Germany for its pork sausage with curry sauce based on spiced ketchup, the so-called "Currywurst". The dish often comes with French fries. Over 800 million curry sausages are consumed annually in Germany, some with hot or very hot sauces added.

Pungency of the various sauces depends on the amount of capsaicinoids which are naturally present in bell peppers or chili peppers. When absorbed by the body, capsaicinoids have the effect of stimulating adrenaline secretion and causing perspiration. Since ancient times, capsaicinoids have been known for their anti-bacterial, stomachic, and body-warming properties. Furthermore, capsaicinoids are very stable elements that maintain their pungency even when used in a variety of cooking methods. The unit used to indicate the pungency of a spice is known as the "Scoville Scale". This scale is indicated in magnitudes based on the amount of time until a taster no longer feels the pungency of a spice extract dissolved in sugar water.

The two main components, capsaicin (69%) and dihydrocapsaicin (22%), are almost twice as strong as the capsaicinoids nordihydrocapsaicin (7%), homodihydrocapsaicin (1%) and homocapsaicin (1%), which are smaller in comparison. Therefore, only capsaicin and dihydrocapsaicin are studied to determine capsaicin levels in the various sauces and pure chili peppers.

Capsaicin

Dihydrocapsaicin

Nordihydrocapsaicin
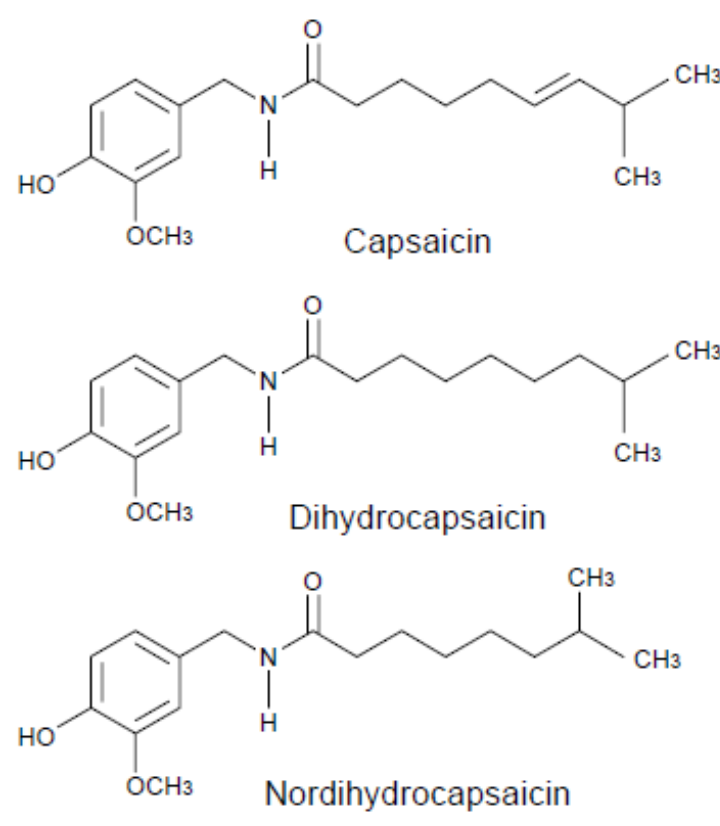
To gain a more objective indicator for the pungency, this poster shows a HPLC methods to analyze the capsaicinoids in chili products.

2. Method Development

To measure the exact content, the Shimadzu i-Series LC-2040C 3D compact HPLC system was used for highspeed analysis. Equipped with a photodiode array (PDA) and fluorescence detector, standards of the two main components were analyzed first.
An isocratic method was developed by using a C18 column and a mixture of 1.0 % acetic acid in water and acetonitrile (60:40 v/v) as mobile phase. Further method parameters are listed in table 1.

First, capsaicin and dihydrocapsaicin standards are tested in different concentrations (5, 10, 25, 50, 75 and 100 µg/mL) to create a calibration curve. Fluorescence detection exhibits approximately 16-fold higher sensitivity than detection with the PDA, as shown in figure 1.
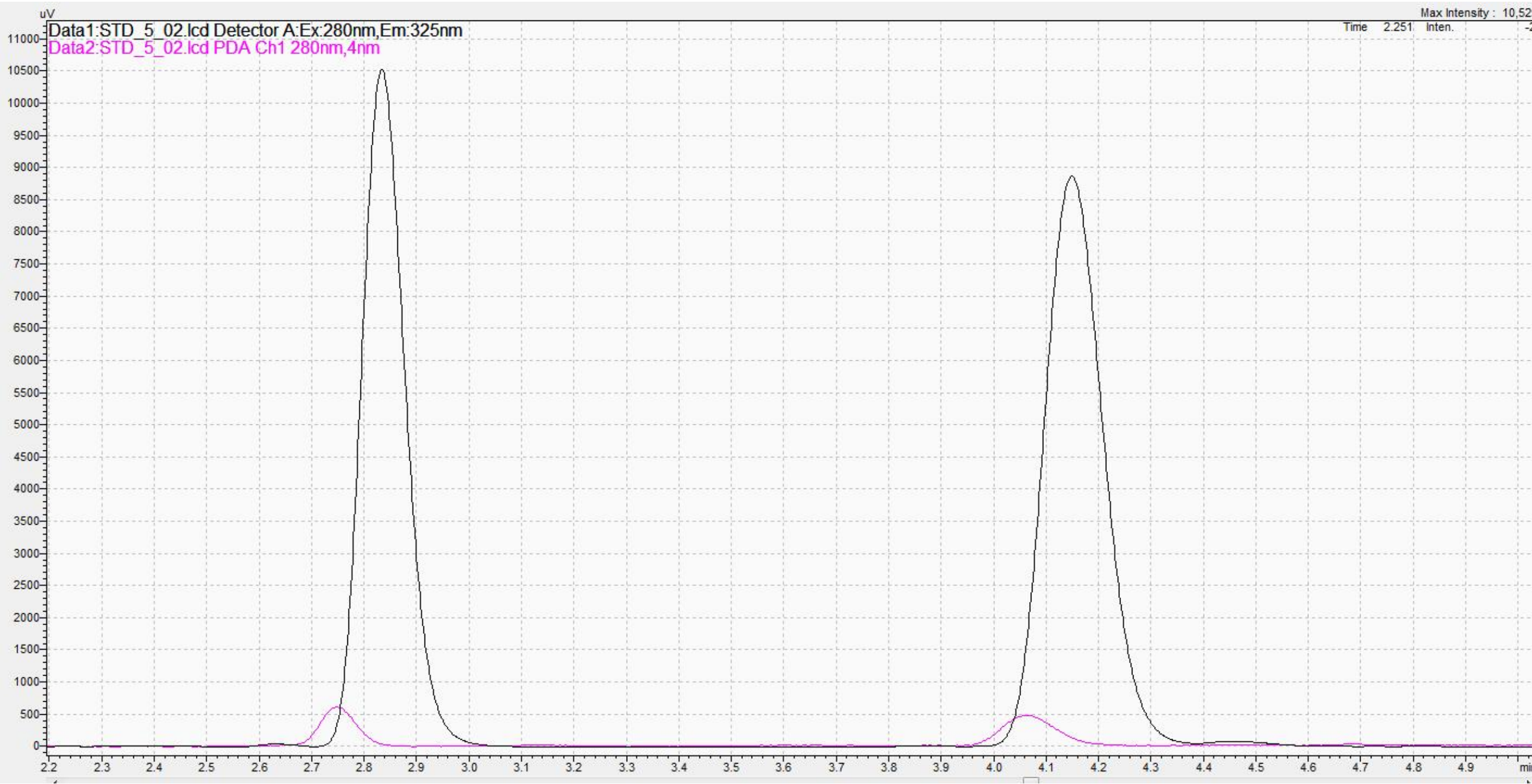

Figure 1: Comparison of RF (black) with PDA detection (violet)

Focus will therefore only be on the fluorescence detector analyses. Capsaicin is the peak eluting at 2.84 min and the dihydrocapsaicin is the peak later eluting at 4.16 min. In addition Nordihydrocapsaicin (RT: 2.58 min) was identified but due to a missing standard of this compound, it was not quantified.

3. Results

The two calibration curves of capsaicin and dihydrocapsaicin (figure 3) serve as a basis for later determination of the capsaicin content in the extracted chili peppers and the chili sauces. For sample preparation of the dried chilies and sauces, a defined amount of sample is first weighed (1 g chili pepper, 2 g sauce) and dissolved in 10 mL ethanol. After one hour of extraction in a water bath (100 ° C) and filtration of the solution, 1 µL is injected (figure 2). In total, four chili peppers and four sauces with distinct degrees of pungency were examined. Figure 4 shows the chromatogram of a sauce with a pungency of level seven, i.e. medium to high, in which capsaicin, dihydrocapsaicin and also nordihydrocapsaicin can be identified.
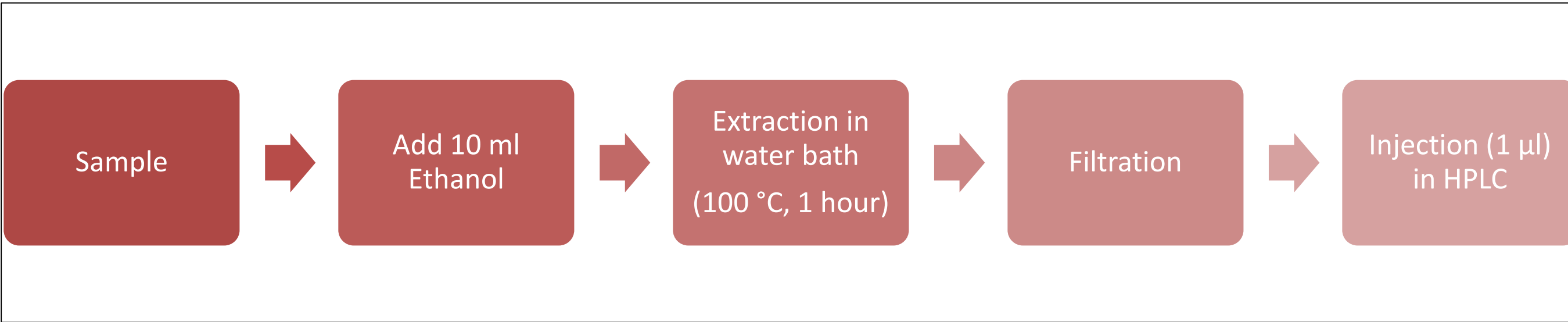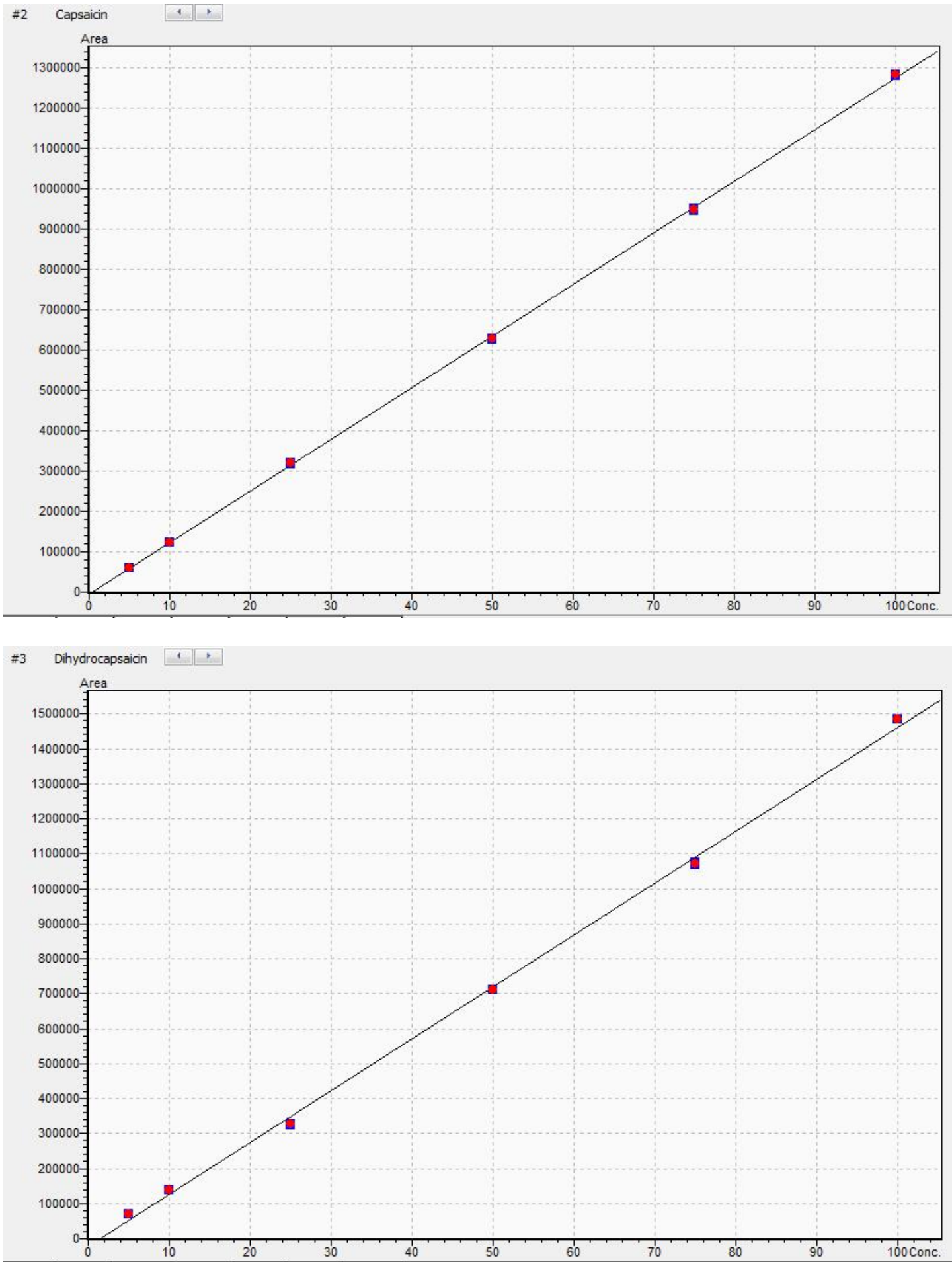

Figure 2: Sample Preparation

Sample → Add 10 ml Ethanol → Extraction in water bath (100 °C, 1 hour) → Filtration → Injection (1 µl) in HPLC

Figure 3: Calibration curves of capsaicinoids

The calibration curves of capsaicin and dihydrocapsaicin are used for quantification. To determine the degree of pungency, the concentrations are ascertained according to the peak areas and converted to the initial weight. The result can then be converted to the Scoville scale using the formula below (without nordihydrocapsaicin) to determine the degree of pungency of chili peppers and sauces.

Formula for the degree of pungency (Scoville)

$$SHV = C + D + N$$

C = (µg capsaicin per gram) × 16,1
D = (µg dihydrocapsaicin per gram) × 16,1
N = (µg nordihydrocapsaicin per gram) × 9,3

Table 1: Method parameters for analysis of capsaicin and dihydrocapsaicin

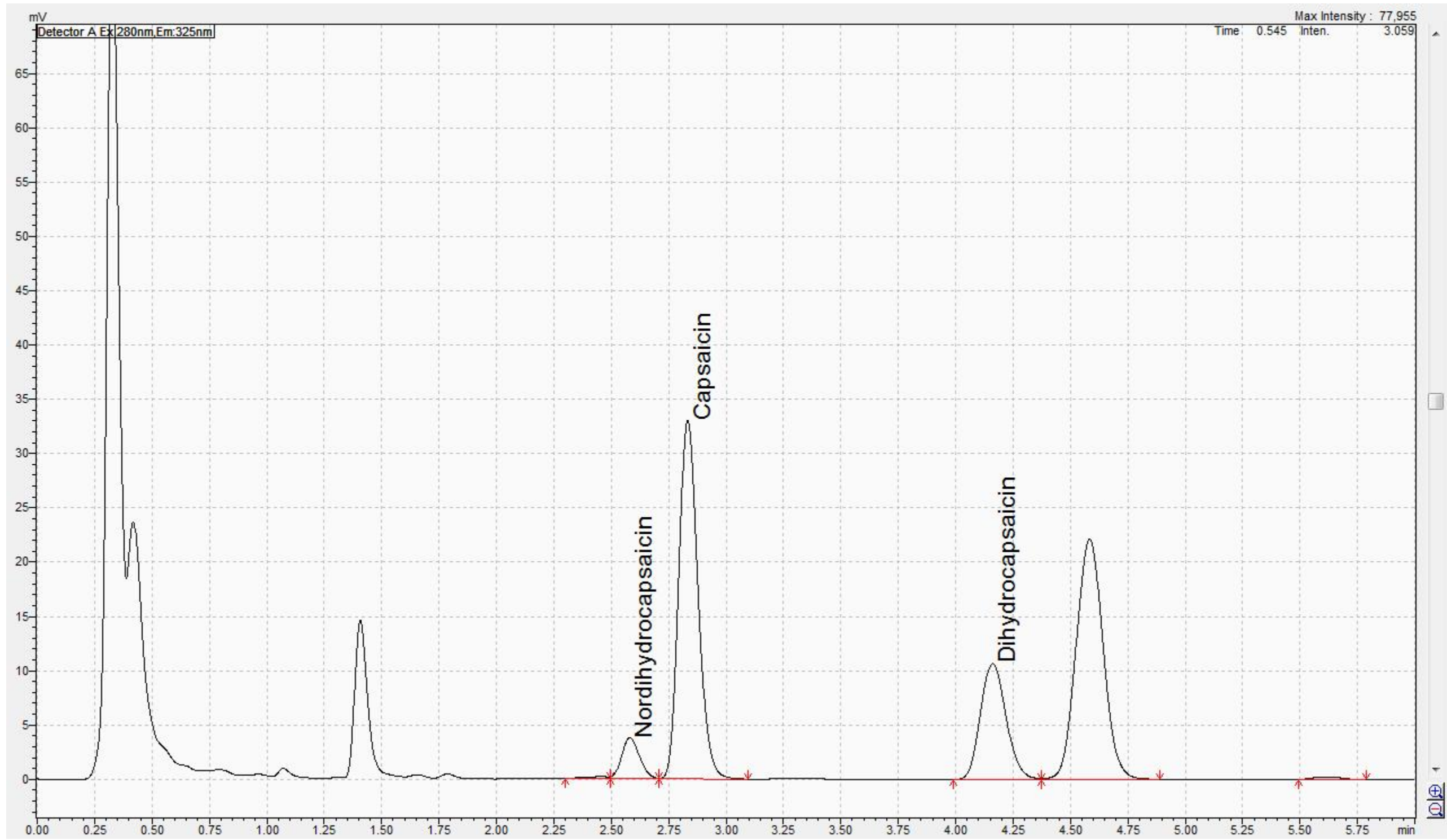|	
Column:	Shim-Pack GIST C18 2.1 x 100 mm; 2µm
Mobile Phase:	A: 1.0 % acetic acid in H_2O; B: Acetonitrile (A:60 Vol.-% B:40 Vol.- %)
Flow rate:	0.9 ml/min
Temperature:	50 °C
Detection:	PDA 280 nm, RF-20Axs: Ex 280 nm, Em 325 nm
Injection Volume:	1 µl

Figure 4: Chromatogram of the sauce with pungency level 7

The results of the measurements of the four hot sauces as well as of the chili peppers are shown in table 2 and 3. As expected, we can see a correlation between the capsaicinoid content and the amount of chilis/chili extract used for the production of the sauces.

Table 2: Results of hot sauces

Sample	Capsaicin	Dihydrocapsaicin	Scoville
	µg/g (sample)	µg/g (sample)	
Sauce 5	6.8	11.6	296
Sauce 7	405.6	120.9	8.478
Sauce 10	1,103.2	442.3	24,882
Sauce 10+	4,867.0	3182.4	129,594

Table 3: Results of chili peppers

Sample	Capsaicin	Dihydrocapsaicin	Scoville
	µg/g (sample)	µg/g (sample)	
Home Grown	1,501.4	388.5	30,427
Habanero	1,693.4	782.2	39,857
Piri Piri	4,899.7	2,273.0	115,125
Bhut Jolokia	24,356.1	8,818.6	534,113

4. Conclusion

The method depicted shows an easy sample preparation and fast analysis of chili products. The results obtained enable an approximate estimation of the expected level of pungency, but due to the non-inclusion of the nordihydrocapsaicin, Scoville results are considered to be too low. As expected, the results reveal a significantly higher degree of pungency of pure chili peppers than for Currywurst sauces. This difference is a result of the same sample processing for both types of samples and the diluting effect of the sauce, which contains components of pure chili peppers. However, the goal was to make the expected trend of pungency visible analytically for sauces with different degrees of pungency, and this has been achieved. This is clearly shown for the examples in table 2.