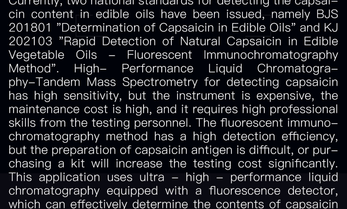
Chromai
- 科诺美 -

Determination of Capsaicin and Dihydrocapsaicin in Edible Oils by Liquid Chromatography Fluorescence Detector



Capsaicin and dihydrocapsaicin are the active components in chili peppers and the main sources of spiciness in chili peppers. Normally, capsaicin or dihydrocapsaicin does not exist in edible vegetable oils. However, the waste cooking oils that have come into contact with chili peppers inevitably contain such components. Therefore, capsaicin-like components can be used as characteristic indicators for identifying gutter oil.

Currently, two national standards for detecting the capsaicin content in edible oils have been issued, namely BJS 201801 "Determination of Capsaicin in Edible Oils" and KJ 202103 "Rapid Detection of Natural Capsaicin in Edible Vegetable Oils – Fluorescent Immunochromatography Method". High- Performance Liquid Chromatography-Tandem Mass Spectrometry for detecting capsaicin has high sensitivity, but the instrument is expensive, the maintenance cost is high, and it requires high professional skills from the testing personnel. The fluorescent immunochromatography method has a high detection efficiency, but the preparation of capsaicin antigen is difficult, or purchasing a kit will increase the testing cost significantly. This application uses ultra – high – performance liquid chromatography equipped with a fluorescence detector, which can effectively determine the contents of capsaicin and dihydrocapsaicin in edible oils, providing a new solution for identifying gutter oil.

Experimental conditions

P40	A10	C10	D50
Quaternary Gradient Pump	+ Automatic Sampler	+ Column Oven	+ Fluorescence Detector

Chromatographic Conditions

Column	Column temperature	Injection volume
Lotus C18 5 μm 4.6*250 mm	30 ℃	10 μL
Excitation/Emission wavelength	**Flow rate**	**Mobile phases**
280 nm 312 nm	1 mL/min	A:Methanol B：Water

Gradient program

Time（min）	A%	B%
0	70	30
14.5	70	30
14.51	95	5
18.0	95	5
18.1	70	30
30.0	70	30

Experimental results

As shown in Table 1, capsaicin and dihydrocapsaicin had a good linear relationship in the concentration range of 0.0625 – 2.5μg/mL, and the correlation coefficients were both 1.0000. The limits of quantification of capsaicin and dihydrocapsaicin were 13.4 ng/mL and 17.06 ng/mL, respectively, and the limits of detection were 4.02 ng/mL and 5.12 ng/mL, respectively.

Table 1. Linear regression equations and correlation coefficients of capsaicin and dihydrocapsaicin

Compound	Concentration range（μg/mL）	Regression equation	Correaltion coefficient
Capsaicin	0.0625–2.5	Y=692.93 X-0.7346	r=1.0000
Dihydrocapsaicin	0.0625–2.5	Y=726.61 X-0.4537	r=1.0000

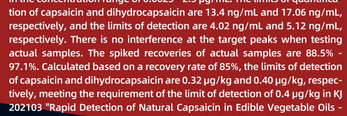


Figure 1. Chromatogram of standard solution of capsaicin and dihydrocapsaicin （0.0625 μg/mL）

Linearity and Sensitivity

The standard solutions of 0.0625 μg/mL, 0.25 μg/mL, and 2.5 μg/mL were injected 6 times respectively, and the RSDs of the peak areas and retention times of each compound were calculated. The results are shown in Table 2. The RSDs of the retention times of each compound at the three concentration levels were all less than 0.5%, and the RSDs of the peak areas were less than 0.8%.

Table 2. Repeatability results of capsaicin and dihydrocapsaicin

Concentration Level	RSD% of Retention Time		RSD% of Peak Area	
	Capsaicin	Dihydrocapsaicin	Capsaicin	Dihydrocapsaici
0.0625 μg/mL	0.41%	0.38%	0.49%	0.80%
0.25 μg/mL	0.09%	0.12%	0.38%	0.58%
2.5 μg/mL	0.32%	0.22%	0.47%	0.48%

Repeatability

Figure 2. Chromatograms of Blank Sample (Blue) and Spiked Sample (Red)

Three concentration levels of 12.5 μg/kg, 25 μg/kg, and 50 μg/kg were added to blank samples, and the spiked recoveries were calculated respectively. The results are shown in Table 3. The spiked recoveries at the three concentration levels were 88.5% – 97.1%.

Table 3. Spiked Recovery Results of Capsaicin and Dihydrocapsaicin

Spiked concentration (μg/kg)	Recovery	
	Capsaicin	Dihydrocapsaicin
12.5	90.7%	91.4%
25	95.7%	97.1%
50	88.5%	91.0%

Recovery

Conclusion

This solution uses the Konome high - performance liquid chromatography - fluorescence detector to determine the contents of capsaicin and dihydrocapsaicin in edible oils. This method has a good linear relationship in the concentration range of 0.0625 - 2.5 μg/mL. The limits of quantification of capsaicin and dihydrocapsaicin are 13.4 ng/mL and 17.06 ng/mL, respectively, and the limits of detection are 4.02 ng/mL and 5.12 ng/mL, respectively. There is no interference at the target peaks when testing actual samples. The spiked recoveries of actual samples are 88.5% - 97.1%. Calculated based on a recovery rate of 85%, the limits of detection of capsaicin and dihydrocapsaicin are 0.32 μg/kg and 0.40 μg/kg, respectively, meeting the requirement of the limit of detection of 0.4 μg/kg in KJ 202103 "Rapid Detection of Natural Capsaicin in Edible Vegetable Oils - Fluorescent Immunochromatography Method". This application provides a new and reliable solution for identifying gutter oil.