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Determination of Wilfordine and Wilforine in honey using Liquid Chromatography with Tandem Mass Spectrometry

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Jianli Chen, Jinting Yao, Taohong Huang, Yuki Hashi
Shimadzu Corporation

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Introduction

Tripterygium wilfordii, which contains a lot of biological toxic compounds such as Wilfordine and Wilforine, is one of the toxic nectar plants. The Wilfordine and Wilforine may be transferred to honey by honey bees. Due to the low content and complex matrix, determination of Wilfordine and Wilforine in honey is not easy. In this study, a highly sensitive method based on liquid-liquid extraction (LLE) and LC-MS/MS has been developed. The results showed that the detection limits of Wilfordine and Wilforine in honey sample were 5.16 and 10.80 ng/kg, respectively.

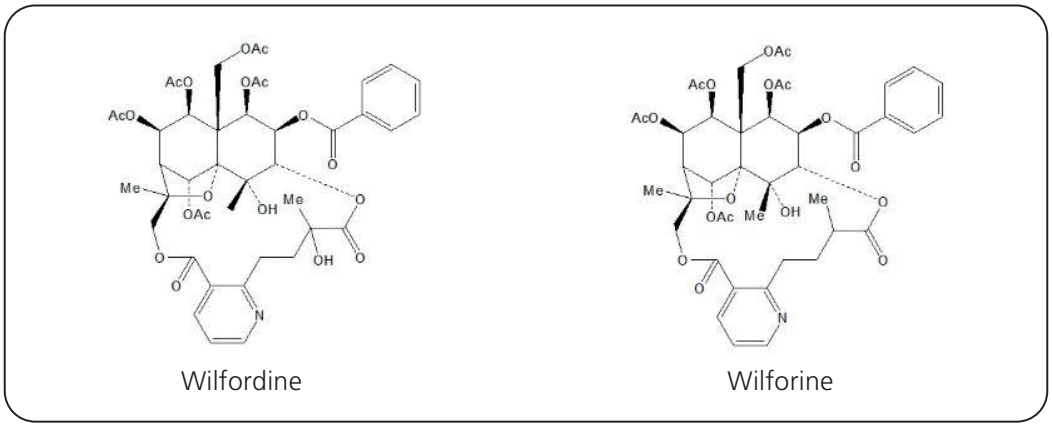

Figure 1 Structure of Wilfordine and Wilforine

Methods

Preparation of Samples

1.0 g of honey sample was added into 10 mL centrifuge tube, and then diluted with 2 mL of pure water. After adding 2 mL of acetonitrile, 0.3 g of NaCl, and 1.2 g of MgSO4 in order, the mixture was vortexed for 2 min and centrifugated at 8000 rpm for 5 minutes. The above solution was withdrawn and filtered (Organic membrane, 0.22 μm) for detection.

Instrucments

The LC-MS/MS system were Prominence LC-20A and triple quadrupole mass spectrometry (Shimadzu Corporation, Kyoto, Japan). Shimadzu LC-20A system consist of a CBM-20A system controller, two LC-20AD pumps, a SIL-20AC autosampler, a CTO-20AC column oven, and a DGU-20A3 online degasser. MS/MS detection was performed by LCMS-8050. Data acquisition and processing were performed with Labsolution software Version 5.72. Electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

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High Speed Mass Spectrometer

Ultra Fast Polarity Switching
- 5 msec

Ultra Fast MRM
- Max. 555 transition /sec

Figure 2　LCMS-8050 triple quadrupole mass spectrometer

Result

Method development for Wilforine and Wilfordine

HPLC conditions

Column : InertSustain C8-3 Column（2.1 mm I.D.×150 mm L., 5 µm）
Mobile phase A : 0.1% formic acid aqueous solution
B : Acetonitrile
Elution Mode : Gradient Elute, the initial concentration of MP B was 30%,

Table 1. LC Time Programme

Time	Module	Command	Value
1.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	90
5.00	Pumps	Pump B Conc.	90
5.10	Pumps	Pump B Conc.	30
10.00	Controller	Stop	

Injection vol. : 10 µL
Column temperature : 35 °C

MS conditions (LCMS-8050)

Ionization : ESI, Positive MRM mode
Nebulizer Flow : 3.0 L/min
Heating Gas Flow : 8.0 L/min
Interface Temperature : 400 °C
DL Temperature : 150 °C
Heat block Temperature : 300 °C
Dry Gas : 12.0 L/min

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Table 2. MRM transition

Compound	MRM transition	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Wilfordine	884.30>856.20*	-12	-25	-30
	884.30>176.10	-12	-50	-18
Wilforine	868.30>178.10*	-12	-60	-18
	868.30>206.10	-12	-43	-20

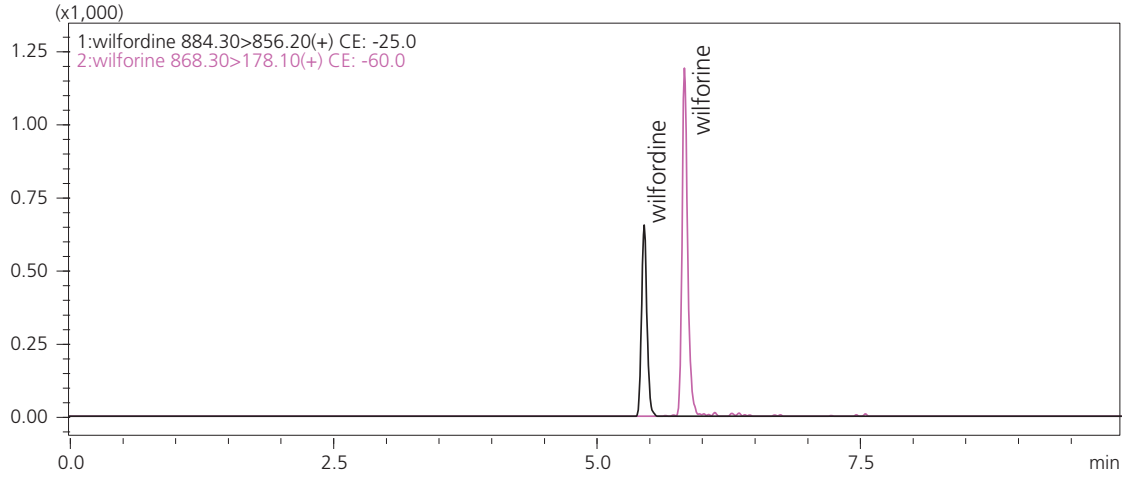

Figure 3 MRM chromatograms of standard solution of Wilforine and Wilfordine (Concentration of each compound were 0.05 ng/mL)

Analytical Performance

Linearity

The determination of Wilfordine and Wilforine were verified using an external standard method. The external calibration was performed by plotting peak area versus concentration of Wilfordine and Wilforine (As seen in Figure 4). The sample solutions were spiked with stock solution to get final concentrations of Wilfordine and Wilforine at 0.01, 0.02, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 ng/mL.

The detailed calibration curves, ranges, correlation coefficients and precisions were shown in Table 2.

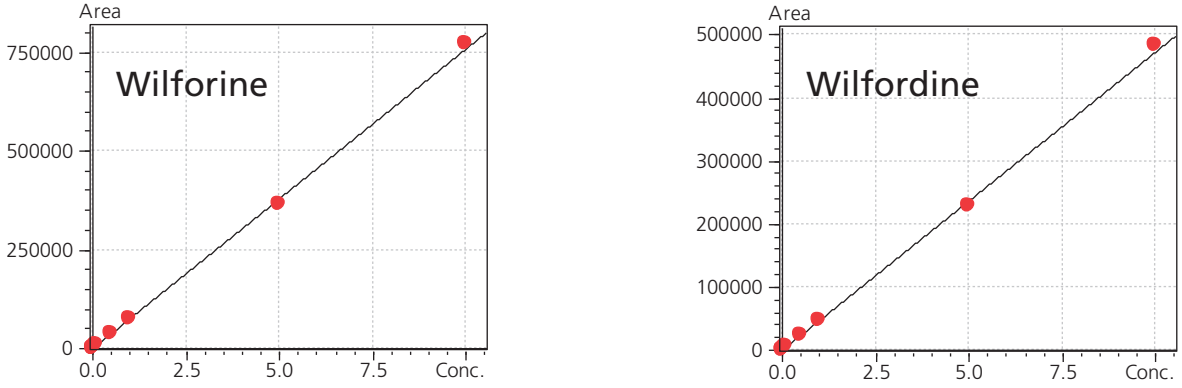

Figure 4 Calibration curve of Wilfordine and Wilforine

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Table 3. Parameters of Calibration Curves

Compound	Calibration Curves	Range (ng/mL)	Coefficient (r^2)	Precision (%)
Wilforine	Y=(75959.6) X -45.2	0.01~10.0	0.9996	92.1~113.8
Wilfordine	Y=(47426.1) X+ 206.6	0.01~10.0	0.9997	87.7~108.3

Sensitivity

Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times of the baseline noise, and the detection limits of Wilforine and Wilfordine were 1.3 and 4.3 ng/L, the quantification limits were 2.7 and 9.0 ng/L, respectively.

Recovery

Preparation of blank honey samples as well as blank honey samples spiked at 0.05 ng/g and 5.0 ng/g. According to the mentioned method before, each sample was measured three times in parallel. The recovery is calculated by subtracting the content of Wilfordine and Wilforine in blank honey samples. The recovery results were shown in table 4.

Table 4. Recovery results

No.	Compound	Spiked at 0.05 ng/g (%)	Spiked at 5.0 ng/g (%)
1	Wilfordine	104.0	99.6
2	Wilforine	116.0	98.8

Conclusions

In this paper, a fast and effective method for the sensitive and reliable analysis of Wilfordine and Wilforine using LC-MS/MS was established.The method has good linearity, with correlation coefficient greater than 0.999, the limit of detection were 1.3 and 4.3 ng/mL, the quantification limits were 2.7 and 9.0 ng/L, respectively. The recoveries were between 98.8~116.0%.

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