

## High Speed Analysis of Piperine in Pepper by Ultra High-performance Liquid Chromatography with Photodiode Array Detection

### Introduction

Piperine is a particular spicy constituent that is contained in Piperaceae plants, which is effective to improve energy metabolism, the circulation of the blood and excessive sensitivity to cold. It is also known that Piperine has an antibacterial action, an antiseptic action and an insecticidal action, and it is contained more in black pepper. In this Application Data, Piperine in Pepper was analyzed by Ultra High-performance Liquid Chromatography with Photodiode Array Detection, which has high speed data sampling capability as many as 100 spectra/sec.

**Keyword :** UHPLC, Pepper, Piperine, 1.8 mm, C18 Column, PDA detector

### Experimental

#### Equipment

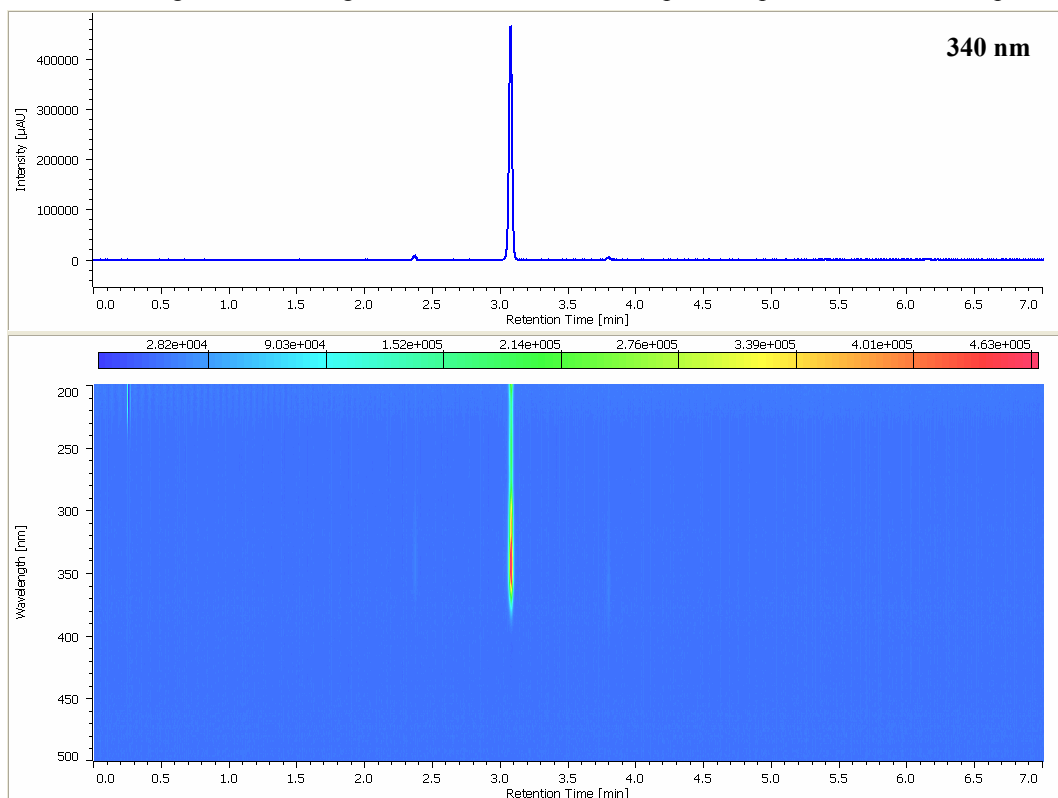
Pump: X-LC 3185PU x 2  
 Degasser: X-LC 3080DG  
 Mixer: X-LC 3180MX  
 Column oven: X-LC 3067CO  
 Autosampler: X-LC 3159AS  
 Detector: X-LC 3110MD

#### Conditions

Column: ZORBAX Eclipse Plus C18 (3.0 mmID x 50 mmL, 1.8  $\mu$ m)  
 Eluent A: 0.1% Formic acid  
 Eluent B: 0.1% Formic acid in Acetonitrile  
 Gradient condition: (A/B), 0 min (70/30)  $\rightarrow$  7 min (10/90)  $\rightarrow$  7.5 min (10/90)  $\rightarrow$  7.55 min (70/30) 1 cycle; 10 min  
 Flow rate: 0.8 mL/min  
 Column temp.: 40°C  
 Wavelength: 200 - 500 nm  
 Injection volume: 1 mL  
 Standard sample: Piperine 0.1 mg/mL in Water/Acetonitrile (90/10)

### Result

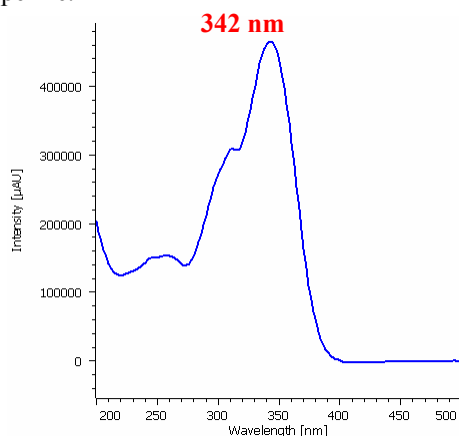
Fig. 1 shows a chromatogram and a contour plot of Piperine standard sample. Longer analysis time was taken as compared to the time using of UHPLC in general, in consideration of Piperine separation in actual sample.



**Fig. 1** Chromatogram and contour plot of Piperine standard sample  
 1: Piperine

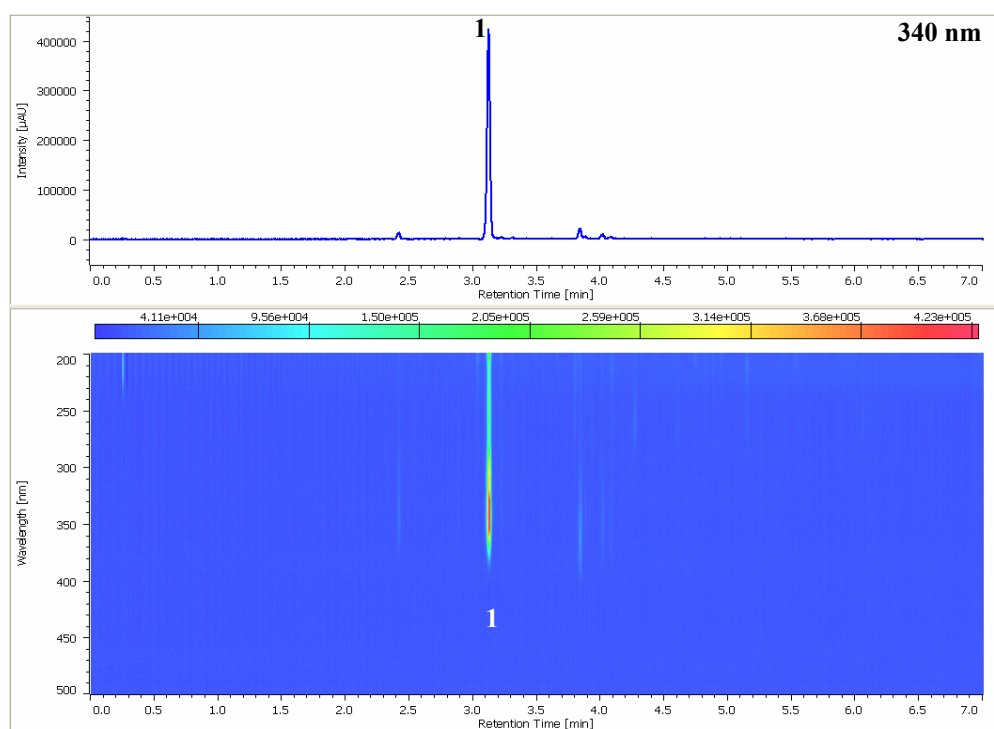
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Fig. 2 shows a spectrum of Piperine.



**Fig. 2** Spectrum of Piperine sample

Fig. 3 shows chromatogram and contour plot of extract of coarsely ground pepper by using Supercritical Fluid Extraction technology. Very sharp peak was obtained.



**Fig. 3** Chromatogram and contour plot of coarsely ground pepper  
1: Piperine

#### Sample preparation :

Coarsely ground pepper was extracted by Supercritical Fluid Extraction system and its extract was dissolved in 1 mL of Acetonitrile and diluted with Acetonitrile to 1/100 concentration. Then it was filtrated using 0.2 mm membrane filter.

#### Super Critical Fluid extraction condition

Sample :	Coarsely ground pepper 1.0 g
Extraction vessel :	10 mL ( Glass wool has been mounted in both IN and OUT
Temperature :	40 °C.
Back Pressure :	20 MPa
CO2 Flow Rate :	3.0 mL/min

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