Application Note
No. 770/2021

Determination of Piperines in pepper

SpeedExtractor E-916:
Extraction of piperines from black and long pepper using the SpeedExtractor E-916

1. Introduction

Pepper is one of the most widely used spice and seasoning. Piperine – the bioactive compound of white, black or long pepper – imparts the spice with its pungency and biting taste. Piperine was found to have therapeutic and medicinal effects due to its anti-oxidant, -inflammatory, -cancer, -tumor, -pyretic properties [1,2,3].

This Application Notes presents an effective method for the determination of piperines from pepper samples reducing the extraction time using Pressurized Solvent Extraction (PSE). The PSE method is carried out on the SpeedExtractor E-916 under elevated temperature and pressure. In comparison to the norm AOAC 987.07 [4] the extraction time can be significantly reduced.

2. Equipment

- SpeedExtractor E-916, equipped with 10 mL cells
- Amber vial 20 mL
- Analytical balance (accuracy +/- 0.1 mg)
- UV/Vis spectrophotometer (Heλios UV Visible, Thermo Electron Corporation)
- Volumetric flasks (100 mL)
- Volumetric pipette (1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL)
- Ultra Centrifugal Mill ZM 200 (Retsch)

3. Chemicals and Materials

Chemicals and consumables:

- Ethanol denaturated, EMSURE® for analysis (denaturated with 1% MEK) (VWR, Order No. 1.00974.2511)
- Piperine ≥ 97% (Sigma-Aldrich, Order No. P49007)
- Quartz sand, 0.3-0.9 mm (BUCHI, Order No. 037689)
- Aluminium foil

For safe handling please pay attention to all corresponding MSDS.

Samples:

- Organic long pepper, expected piperine content: 4-5% [5]
- Black pepper, expected piperine content: up to 9% [5]
- Organic black pepper, expected piperine content: up to 9% [5]

All samples were purchased in a local supermarket. The samples were homogenized to a powder using the Ultra Centrifugal Mill.

4. Procedure

The determination of piperins from pepper samples includes the following steps:

- Preparation of the cells
- Extraction of the samples
- Preparation of standard solutions
- UV/Vis spectrophotometry
- Calculation of extractable content

4.1 Preparation of the cells

1. Place a cellulose bottom filter and a metal frit into the bottom of a 10 mL extraction cell and close it with a plug screw. Place a 10 mL funnel on top of the cell.
2. Add 1 g quartz sand to each cell.
3. Weigh approximately 0.5 g sample into a 20 mL amber vial, note down the exact amount. Add approximately 9 g quartz sand to the vial and mix it well with a spatula. Add the mixture to the extraction cell using the funnel.
4. Rinse vial and funnel with additional quartz sand – ensure to only fill the extraction cell up to 1 cm below bearing surface.
5. Place a top cellulose filter into the extraction by using the plunger.

4.2 Extraction of the samples with the SpeedExtractor E-916

6. Preheat the SpeedExtractor E-916 to the temperature of the method (see Table 1).
7. Protect the collecting vessels from light – e.g. by covering it with aluminium foil[1].
8. When the heating block of the SpeedExtractor E-916 has reached the temperature, place the extraction cells in using the gripper.

Figure 1: Due to possible photodegradation of piperine, the extraction was run under opaque conditions.

9. Extract the samples with the SpeedExtractor E-916 using the method given in Table 1.

Table 1: Extraction parameters for SpeedExtractor E-916

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	Ethanol 100%
Cells	10 mL
Vials	240 mL
Cycles	2
Heat-up	1 / 1 min
Hold	10 / 2 min
Discharge	2 / 2 min
Flush with solvent	1 min
Flush with gas	2 min

10. Transfer the extract from the collection bottle into a 100 mL volumetric flask. Residues in the collecting bottle are rinsed with additional ethanol before filling up the volumetric flask to the 100 mL mark. This is the extract solution.

[1] Instead of covering the collection vessels with aluminium foil it is also possible to use amber collection vessels.

4.3 Preparation of standard solutions

11. Weigh 100 mg of piperine into a 100 mL volumetric flask, dissolve and dilute to mark with ethanol. Note the exact weight! This is the stock solution.
12. Transfer 10 mL of the stock solution into a 100 mL volumetric flask and dilute to volume.
13. Transfer 1, 2, 3, 4, 5, 6 mL aliquots into six different 100 mL volumetric flasks, fill each up to volume with ethanol.

These standard solutions contain (depending on the exact weight) 1, 2, 3, 4, 5, 6 mg/L piperine for the 1, 2, 3, 4, 5, 6 mL aliquots, respectively.

4.4 UV/Vis spectrophotometry

14. Sample solution: Transfer 2.0 mL of the extract solution into a 100 mL volumetric flask and fill up with ethanol to the mark.
15. The absorbance of the sample and standard solutions is determined and compared to the pure ethanol at a wavelength of 342-345 nm.

4.5 Calculation

The correlation between the concentration and absorptivity of the piperines can be obtained by equation (1):

$$Absorptivity\ Piperines, A = \frac{a_n}{d * c_n} \quad (1)$$

A: Absorptivity of piperines at 344 nm in L/(mg*cm)
a_n: absorbance of standard solution *n* at 344 nm
d: pathlength (1 cm)
c_n: concentration of standard solution *n* in mg/L

This correlation can be applied to the measured sample solutions to determine the corresponding concentration (see Chapter 5). The percentage content of piperines is calculated according to the following equation (2).

$$\%\ Piperines = \frac{c_s * 0.5}{m_{sample}} \quad (2)$$

% Piperines: Percentage of piperine content in the sample
m_{Sample}: Sample weight [g]
c_s: concentration of piperine in sample solution *s* in mg/L
0.5: factor due to the dilution of the extract

5. Result

The standard solutions were analysed using the UV/Vis spectrophotometer. The correlation between the concentration and the absorbance was determined with a linear regression, which is only applicable for the range covered by the standard solutions.

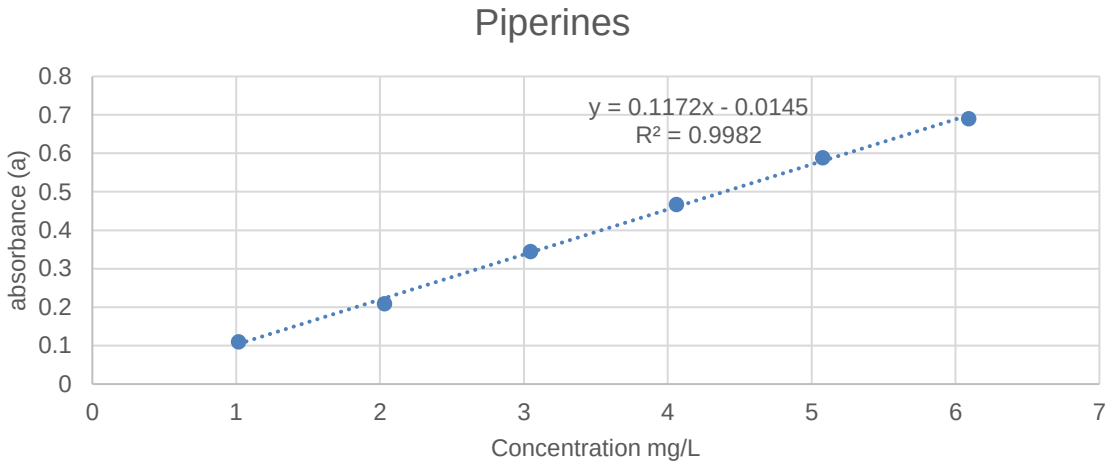

Figure 2: Linear regression of the standard solutions.

For the piperine determination, the samples were extracted and analysed in triplicates. The results are shown in Table 2.

Table 2: Determined piperine content in long and black pepper

Sample	m_{sample} [g]	Absorption a	Piperines [%]	Mean value
Organic long pepper	0.497	0.501	4.43	**4.38%** rsd : 1.24%
	0.5036	0.503	4.39	
	0.5123	0.504	4.32	
Black pepper	0.4949	0.402	3.59	**3.55%** rsd : 1.03%
	0.5115	0.408	3.53	
	0.5032	0.402	3.53	
Organic black pepper	0.5032	0.496	4.33	**4.37%** rsd : 0.72%
	0.5082	0.508	4.39	
	0.5023	0.501	4.38	

The determined piperine content of 4.38% for the long pepper sample corresponds well to the expected value of 4-5%. The expected piperine content of (organic) black pepper has a wide range and depends strongly to the origin [1]. The organic black pepper was found to have higher piperine content than normal black pepper. The relative standard deviations are low, indicating a complete extraction.

6. Conclusion

In the official method AOAC 987.07, the sample was dispersed in 70 mL ethanol and heated under reflux, while it was protected from light for 1 h. After extraction, the suspension was filtered through a filter paper and the solid residues washed with ethanol into a 100 mL volumetric flask. The filtration step is omitted with the SpeedExtractor E-916, since the extract in the collecting vessel is easily transferred into a 100 mL volumetric flask using a small amount of solvent.

The determination of piperine content in long and black pepper by use of the SpeedExtractor E-916 provides reliable and reproducible results. Compared to the official method AOAC 987.07, the timeconsuming filtration step after the extraction is omitted using the SpeedExtractor E-916.

7. References

[1] Gorgani, L. et al, Piperine – The Bioactive Compound of Black Pepper: From Isolation to Medicinal Formulations, Comprehensive Reviews in Food Science and Food Safety, 16, 124-140, 2017.
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[3] Lee, E. B. et al, Pharmacological study on piperine, Arch. Pharm. Res., 7, 127-132, 1984.
[4] AOAC 987.07 Piperine in Pepper Preparations
[5] Tiwari, A et al, Piperine: A comprehensive review of methods of isolation, purification, and biological properties, Medicine in Drug Discovery, 7, 1-21, 2020.