Application Note
No. 765/2021

Determination of Piperine in pepper

UniversalExtractor E-800
Extraction of piperine in pepper using the UniversalExtractor E-800

1. Introduction

A simple and reliable procedure for the determination of the piperine content in long and black pepper is introduced.

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].

In the presented application, the sample is extracted with the UniversalExtractor E-800 using the Hot extraction method. Due to possible photodegradation of piperine [4], the UniversalExtractor E-800 had to be run under opaque conditions. The total piperine content is determined using UV/Vis spectrophotometry. The procedure complies with the official method of AOAC 987.07.

2. Equipment

- UniversalExtractor E-800 with chamber heater
- Analytical balance (accuracy ± 0.1 mg)
- UV/Vis spectrophotometer (Helios UV Visible, Thermo Electron Corporation)
- Volumetric flasks (100 mL)
- Volumetric pipette (1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL)
- Rotavapor R-100

3. Chemicals, Reagents and Materials

Chemicals:

- Ethanol denaturated, EMSURE® for analysis (denaturated with 1% MEK) (VWR, Order No. 1.00974.2511)
- Glass wool
- Cellulose thimbles, 33x150 mm, BUCHI (Order No. 11067446)
- Piperine ≥ 97% (Sigma-Aldrich, Order No. P49007)
- Aluminium foil, purchased in a local supermarket

For a safe handling please follow the guideline mentioned in the corresponding MSDS!

Sample:

- 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 bought from a local supermarket.
The samples were mixed to a powder.

4. Procedure

The determination of the piperine content includes the following steps:

- Preparation of standard solutions
- Direct extraction of pepper powder
- Determination of piperine content using UV/Vis spectrophotometry

4.1 Preparation of standard solutions

1. Weigh 100 mg of piperine into a 100 mL volumetric flask, dissolve and dilute to mark with ethanol. Note the exact weight!
2. Transfer 10 mL of the stock solution into a 100 mL volumetric flask and dilute to volume.
3. 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.

4.2 Weighing of the samples

4. Place the cellulose thimble into a thimble holder.
5. Weigh 0.5 g of homogeneous sample into the cellulose thimble. Note the exact weight.
6. Use the glass wool to cover the sample inside the cellulose thimble.

4.3 Extraction of piperines with the UniversalExtractor E-800 Hot Extraction

4.3.1 Preparation of the beakers

7. Always use clean beakers for the extraction.

4.3.2 Hot Extraction

8. Place the cellulose thimbles containing the sample into the extraction chamber and adjust the level sensor to the sample's height.
9. Fill the solvent into the beakers and place them on their corresponding heating plate.
10. Close the protection shield and lower the rack. Alternatively, fill the solvent through the condensers after lowering the rack. Activate the occupied positions and open the cooling water tap or switch on the connected chiller.
11. Envelope the protection shields and from the top with aluminium foil to operate under opaque conditions.

Figure 1: The extraction of the samples was run under opaque conditions

12. Start the Hot extraction according to the parameters listed in Table 1.

Table 1: Parameters for the Hot Extraction with the UniversalExtractor E-800.

Step	Value	Heating level
Extraction method	Hot extraction	
Solvent	Ethanol	
Extraction	1 h	Extraction:18[1] Chamber: 9[2]
Rinse	10 min	18
Drying	☑ AP	11
Solvent volume [mL]	100	

4.3.3 Post Extraction

13. The extract is transferred into a 100 mL volumetric flask. Residues in the beaker are rinsed leelll with additional ethanol before filling up the volumetric flask to the 100 mL mark (= extract solution.

Note: For the recovered solvent: Before re-usage, check the solvent for piperine impurities by determining the absorbance and compare it to the pure solvent. In case of impurities, the Soxhlet chamber must be cleaned by either disassembling and manual rinsing or by starting a cleaning method (e.g. rinsing for 30 minutes) with pure solvent. The impure solvent can be collected and purified by distillation using for example a Rotavapor R-100.

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 solution is determined and compared to the pure ethanol at 342-345 nm.

4.4.1 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
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 then calculated according to the following equation (2).

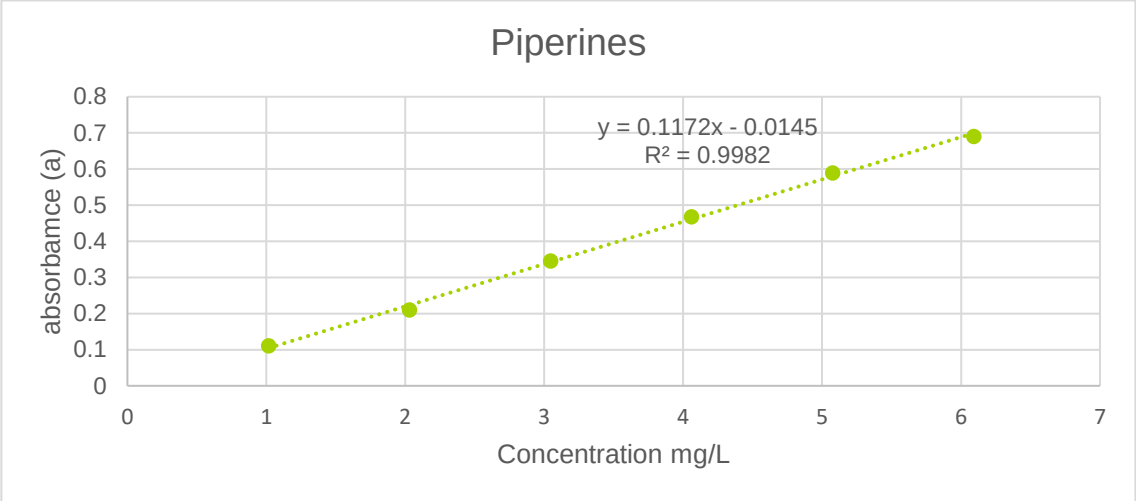
$$\%\ Piperines = \frac{c_s * 0.5}{m_{sample}} \quad (2)$$

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

[1] Heating level proposed by the system depending on the selected solvent.
[2] The heating level for the chamber heater was higher than proposed to reach an intense boiling/reflux as described in the official AOAC method.

5. Results

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.



For the piperine determination, the samples were 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	Piperines [%]	Mean value
Long pepper	0.5324	0.511	4.21	**4.22%** rsd : 0.18%
	0.5045	0.483	4.21	
	0.5189	0.499	4.22	
Black pepper	0.5133	0.402	3.46	**3.45%** rsd : 2.80%
	0.5382	0.408	3.35	
	0.5475	0.440	3.54	
Organic black pepper	0.5096	0.497	4.28	**4.28%** rsd : 1.52%
	0.5219	0.501	4.22	
	0.5131	0.508	4.35	

The determined piperine content of the long pepper sample corresponds well with 4.22% to the expected value of 4-5%. The expected piperine content of (organic) black pepper has a wide range and depends strongly on the origin. The tested organic black pepper was found to have higher piperine content than normal black pepper.

6. Conclusion

In the method described in AOAC 987.07, the sample was dispersed in 70 mL ethanol and heated under reflux and protected from light for 1 h. A similar process is obtained with the E-800 Hot Extraction. As mentioned in the standard procedure, after the extraction the suspension was filtered, and the solid residues washed with ethanol into a 100 mL volumetric flask. This filtration step can be omitted with the E-800 Hot Extraction method, since the extract in the beaker is easily transferred into a 100 mL volumetric flask with a small amount of solvent.

Determination of piperine content in long and black pepper by use of the UniversalExtractor E-800 provides reliable and reproducible results. As compared to the method described in AOAC 987.07, the laborious filtration steps after the extraction step in the piperine determination are omitted using the BUCHI automated extraction system.

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.
[2] Mujumdar, A. M. et al, Anti-flammatory activity of piperine, Jpn. J. Med. Sci. Biol., 43, 95-100, 1990.
[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.

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Operation Manual of the UniversalExtractor E-800