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STABILITY OF THIOAMIDE TYPE OF PIPERINE UNDER ACIDIC AND BASIC CONDITIONS

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KEYWORDS

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Acidic and basic conditions

Degradation

Chromatography

Cancer

ABSTRACT

Thioamide type of piperine, "thiopiperine" is a derivate of piperine that having high potency against breast cancer cells. This research was intended to evaluate the stability of thiopiperine under highly acidic and basic conditions for 72 h at 60ºC. This study was conducted by the SHIMADZU® UFLC system integrated with a PDA detector, while the analysis was performed in an isocratic separation mode using column C-18 (COSMOSIL®), 150 mm x 4.6 mm, column particle size: 5 µm. Chromatography condition was set using a mobile phase consisting of 50% aqueous acetonitrile with a flow rate of 1 mL/min, while the detection wavelength was 340 nm. The result showed that thiopiperine undergoes extensive degradation under acidic and basic environments.

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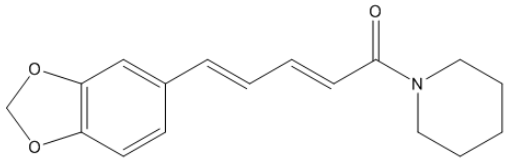
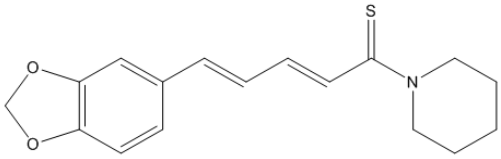
1 Introduction

Piperine, an alkaloid compound derived from pepper (*Piper nigrum* L.), has various pharmacological effects such as anti-inflammatory, anticancer, antiviral, anti-allergic, anti-epileptic, anti-depressant, and anti-Alzheimer's (Shinwari et al., 2019; Stojanović-Radić et al., 2019). Further, it also magnifies the bioavailability of several drugs such as midazolam, diclofenac, resveratrol, isoniazid, domperidone, simvastatin, verapamil, and beta-lactam antibiotic (Tiwari et al., 2020). Structurally piperine has an amide skeleton that can be modified to optimize its pharmacological activities or minimize its adverse effects. Derivatization of piperine has been reported with various analogs and it has various pharmacological effects such as anti-fungal (Souza et al., 2021), PPARγ agonists (Wang et al., 2020), MAO-B inhibitors (Chavarria et al., 2020), anti-neuroinflammatory (Shahbazi et al., 2020), larvicidal (Tantawy et al., 2020), and antitumor (Rifai et al., 2016; Ferreira et al., 2020).

Due to the high medicinal potential of piperine, recently, a derivative compound thioamide-type of piperine (thiopiperine) had been synthesized by modifying the structure of piperine from black pepper. The compound was modified by converting the carbonyl group to a sulfur analog (thiocarbonyl) using *lawesson's* reagent (Figure 1). Aswad (2019) suggested that this compound shows activity against 4T1 breast cancer cells, therefore, this compound can be potentially used in the development of cancer treatment drugs.



a



b

Figure 1 Structure of piperin (a) and Thioamide-type of piperine (b)

In the manufacturing of any drug, data about its stability as candidate active pharmaceutical ingredients must be considered, because it will affect various qualities such as safety and efficacy before it becomes a drug product. According to ICH guidelines, the quality of drug substances and drug products changes with storage time and is influenced by several stress conditions such as acidic, basic, peroxide, temperature, and lights. Furthermore, the determination of stability has only focused on drug products, while data on the stability of the active pharmaceutical ingredients (API) is still less (Blessy et al., 2014). Therefore, this research aimed to determine the stability of thiopiperine as a candidate of API under highly acidic and basic environments.

2 Materials and Methods

2.1 Materials

The materials used in this study are black pepper, piperine (Wako, Japan), acetonitrile (Merck), water pro-HPLC (Merck), Lawesson's Reagent (Wako, Japan), Tetrahydrofuran (THF) (Merck), Dichloromethane (DCM) (Merck), NaOH (Sigma-Aldrich), HCl (Merck). All of the chemicals were used without further purification.

2.2 Synthesis of thiopiperine

Thiopiperine was prepared by the semi-synthesis method from black pepper (*Piper nigrum*) as suggested by Aswad (2019). Ground black pepper (10 g) was refluxed in DCM for 30 minutes followed by evaporation of the solvent *in vacuo*. Cold ether was applied to obtain crude piperine as pale brown solid (126 mg). Subsequently, crude piperine, Lawesson's reagent (162 mg), and 2 mL THF were added into the round bottom flask. The mixture was stirred at room temperature for 24 h. After evaporation of the solvent, the product was purified by flash column chromatography with solvent DCM: EtOAc (2:1) to generate thiopiperine (29 mg) as a bright orange solid.

2.3 Preparation of stock solution

1 mg of thiopiperine was dissolved in 10 mL of acetonitrile to make a solution of thiopiperine (100 µg/mL). In addition, 2.5 mL of stock solution (100µg/mL) was placed into a 5 mL volumetric flask, and then acetonitrile was added up to a 5 mL line (50 µg/mL).

2.4 Instrumentation and chromatographic conditions

HPLC analysis was performed on UFLC Shimadzu® with a PDA detector (λ = 340 nm). The separation was conducted on a C-18 column (Cosmosil®, 5 µm, 4.6x150 mm) with aqueous acetonitrile (50% acetonitrile) as a mobile phase. The flow rate was set 1 mL/minute, injection volume was 20 µL, while analytical run time was 20 min.

2.5 Stability stress testing

2.5.1 Acidic degradation

100 µL of stock solution was placed into a vial, then 900 µL of 0.1 N HCl was added (5 µg/mL). The solution was mixed thoroughly, after that it was stored at 60°C for 72 h. Sampling was conducted at 24 h and 72 h by transfer 100 µL of solution into vial then the volume was adjusted with 900 µL of acetonitrile. The prepared mixture was analyzed by HPLC.

2.5.2 Alkaline degradation

Alkaline degradation was prepared as similar to acidic degradation's procedure, in this 0.1 N NaOH was utilized instead of 0.1 N HCl as a degradation agent.

3 Results and Discussion

Forced degradation study is the process of determining stability that involves degradation of drug substances or drug products at conditions more severe than accelerated conditions, in this case, forced degradation studies help in generating degradants in a shorter period, mostly 2 weeks (Teasdale et al., 2018). To quantify the number of degradants within the forced degradation study, an integrated HPLC system was applied as a stability-indicating method. HPLC method can separate, detect and quantify chemical compounds and various drug-related degradants that are possibly derived during the manufacturing or storage, it can also detect any drug-related impurities that may be introduced during the synthesis (Abdelwahab et al., 2019).

Before stress testing, initially, the HPLC system was calibrated for degradation analysis study. The system suitability testing is applied to verify an analytical method that is appropriate for its intended purpose. Figure 2 exhibited HPLC's chromatogram of piperine and thiopiperine that revealed very high-resolution peaks. Piperine appeared at 3.9 minutes while thiopiperine emerged at 16.6 minutes. The analysis was arranged in an isocratic method mode in column C-18 (150 mm x 4.6 mm) with mobile phase of 50% of aqueous acetonitrile with detector PDA at 340 nm.

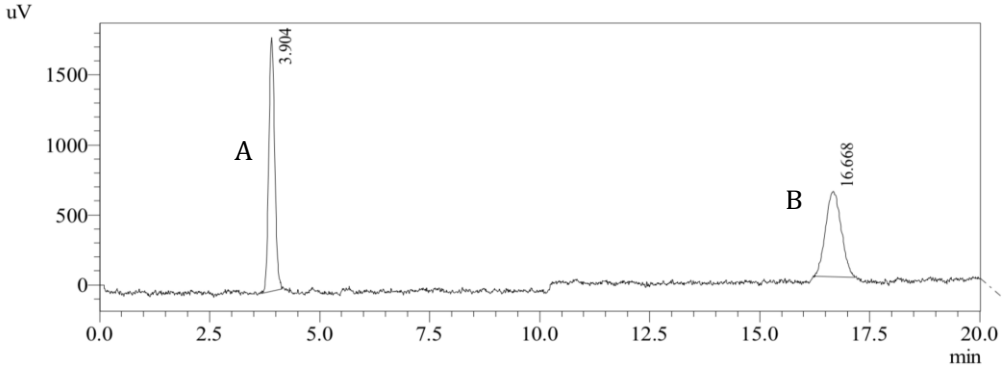


Figure 2 Chromatogram of piperine (A) and thiopiperine (B) on UFLC Shimadzu®, C-18 column (Cosmosil®, 5-µm, 4.6 mm x150 mm) with a mobile phase acetonitrile:water (50:50 v/v) at flow rate 1 mL/min.

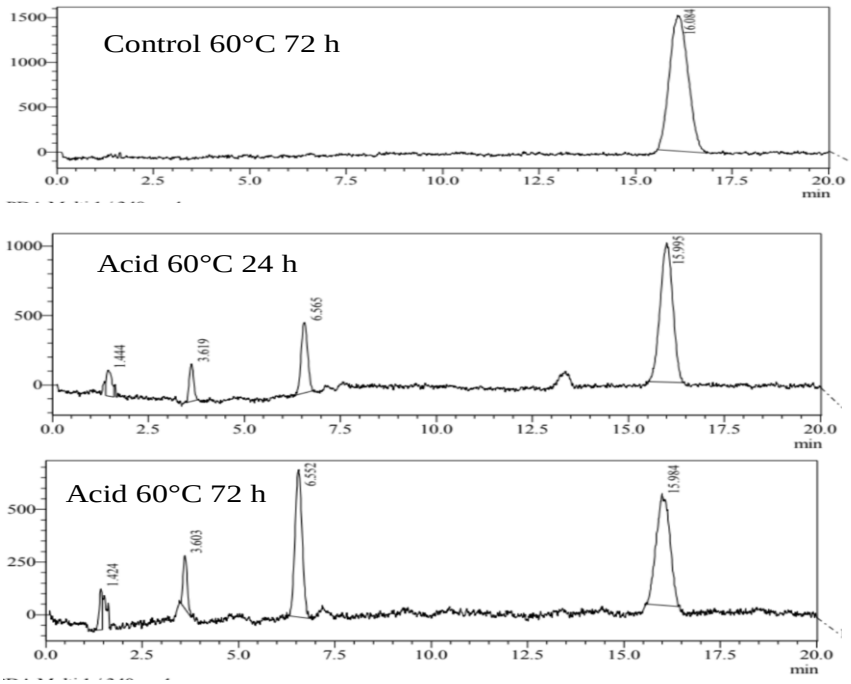


Figure 3 Chromatogram of thiopiperine in 0.1 HCl at 60°C for 24 and 72 h

The data indicated that thiopiperine is more lipophilic than piperine, consider the greater energy of the thiocarbonyl's π-orbital than carbonyl resulting in the electronegativity of thiocarbonyl is less than carbonyl. On the other hand, the polarity of carbonyl is slightly superior to thiocarbonyl (Abboud et al., 1993).

Stress testing of thiopiperine was accomplished in the existence of strong acid (0.1 N HCl), and strong base (0.1 N NaOH) at 60°C for 72h. For acid treatment, thiopiperine solution in 0.1 N HCl environment exhibited degradation process occurred within 24 h after contact with strong acid.

Figure 3 showed that degradants were generated due to the acid environment indicated by new peaks appeared at 1.4 min, 3.6 min, and 6.5 min within 24 h of treatment. In addition, the peak area increased with time. Subsequently, the main peak around 16.0 min was gradually degraded by the time. About 55% of thiopiperine was decayed within 24 h then extensive degradation was occurred up to 75% within 72 h.

Similar to acidic hydrolysis, thiopiperine exhibited a strong degradation under an alkaline environment also (Figure 4). New peaks appeared at 2.3 min, 2.6 min, 3.6 min, and 6.5 min when the thiopiperine was exposed with aqueous NaOH (0.1 N) for 24 h at 60°C. The main peak (16.0 min) was steadily diminished by about 49% within 24 h and decreased 70% within 72 h. In both acidic and basic conditions, thiopiperine underwent a degradation process with time. However, the compound might decay rapidly under acidic conditions than the basic environment.

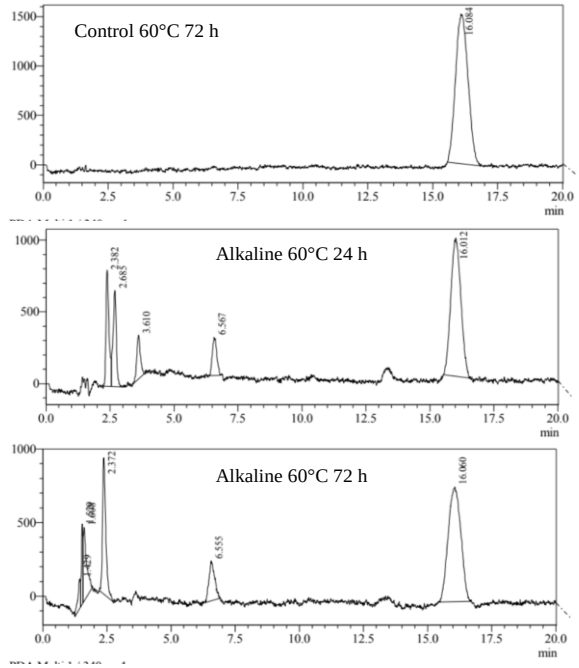


Figure 4 Chromatogram of thiopiperine in 0.1 NaOH at 60°C for 72 h

Conclusion

The stability of thiopiperine investigated through the stability-indicated HPLC method exhibited that the compound was unstable after stress testing for 3 days at 60°C in acidic and basic conditions. The compound was likely to degrade faster under acidic than the basic environment.

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Conflict of Interest

The authors declare that there is no conflict of interest

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