Forced degradation study of sodium nitrate solution and its formulation

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Introduction
Nicotine dependence or tobacco dependence is an addiction to tobacco products caused by nicotine containing in tobacco. Cigarette smoking is the most common type of tobacco use. Recently, there are approximately 1.3 billion smokers around the world. About 84% of them are in developing countries. Approximately 10 million people are killed by tobacco in 2020 if the recent smoking trend continues¹. In 2015, the National Statistical Office of Thailand reported statistics on smoking behavior in 2014 that 20.7% (or 11.4 million) of Thai population who are 15 years old and over are tobacco smoker. Moreover, 10 million of this group (18.2%) are regular smokers². Tobacco addiction is a second-leading cause of death worldwide. The important causes of tobacco-related mortality are atherosclerotic vascular disease, cancer, and chronic obstructive pulmonary disease¹. The 0.5% sodium nitrate mouthwash is used as an aid for smoking cessation in Thailand, which the using of 0.5% sodium nitrate mouthwash is found in some publications³ ⁴. Forced degradation studies are known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc⁵. The stress testing data is useful in understanding the stability of drug during manufacturing, storage, shipping, and patient use⁶. However, stress tests of sodium nitrate solution and its formulation are not reported elsewhere. Thus, the aim of this work was to study the forced degradation of sodium nitrate solution and sodium nitrate mouthwash for smoking cessation. Five topics were investigated i.e. acid hydrolysis, alkaline hydrolysis, thermal hydrolysis, oxidation, and photo-degradation.

Methods
Acid hydrolysis: The 0.2 mL of 5 mg/mL sodium nitrate aqueous solution and 5 mg/mL sodium nitrate mouthwash for smoking cessation were added into the 10-mL volumetric flask. Then, it was diluted with 1 N hydrochloric acid to the volume (n=3), which final concentration of sodium nitrate was 0.1 mg/mL. The obtained solution was filtered using 0.45 µm pore size syringe filter, stored at room temperature for 24 hours, neutralized to avoid further decomposition, and then injected into HPLC instrument for analysis of sodium nitrate remaining in the solution. The sodium nitrate remaining was compared to sodium nitrate content at an initial time.
Alkaline hydrolysis
For alkaline hydrolysis, the procedure was similar to acid hydrolysis but 1 M sodium hydroxide was used instead of 1 M hydrochloric acid.

Thermal hydrolysis
For thermal hydrolysis, the procedure was similar to acid hydrolysis but the ultrapure water was used instead of 1 M hydrochloric acid. Furthermore, the obtained solution was heated at 70 °C for 24 hours before injecting into HPLC instrument.

Oxidation
For oxidation, the procedure was similar to acid hydrolysis but 3% hydrogen peroxide was used instead of 1 M hydrochloric acid.

Photo-degradation
For photo-degradation, the procedure was similar to acid hydrolysis but the ultrapure water was used instead of 1 M hydrochloric acid. Furthermore, the obtained solution was stored under fluorescent and ultraviolet light for 24 hours before injecting into HPLC instrument.

HPLC condition
Analysis was performed using HPLC instrument equipped with photodiode array detector (Agilent 1260 infinity, Agilent Technologies, USA). The separation was done on ZORBAX Eclipse Plus C18 (100×4.6 mm, i.d., 3.5 µm) column with the isocratic system. The 0.01 M octylammonium orthophosphate (pH 7.0) in 30% methanol was used as mobile phase. The mobile phase flow rate was 0.8 mL/min. The column temperature was controlled at 25 °C. The Injection volume was 10 µL. The quantitation wavelength was set at 213 nm.

Results
Forced degradation of sodium nitrate was investigated in solution and formulation form. HPLC chromatograms of sodium nitrate at the initial time point without any forced degradation are shown in Figure 1A. The peak of sodium nitrate was found at a retention time of approximately 3.2 minute. However, a slight shift of peak of sodium nitrate was found in alkaline hydrolysis condition (Figure 1C). A small peak found in the formulation after the peak of sodium nitrate was not identified. It might be the peak of methylparaben and propylparaben containing in the formulation. However, this peak disappeared in acid hydrolysis condition (Figure 1B). In addition, the peak before sodium nitrate peak (Figure 1E) was the peak found in blank hydrogen peroxide solution.

Figure 1. HPLC chromatograms of sodium nitrate solution and its formulation which A was at initial and B to F were after acid hydrolysis, alkaline hydrolysis, thermal hydrolysis, oxidation, and photo-degradation, respectively.

Results from five stress conditions indicated that they did not decrease the stability of sodium nitrate in solution and its formulation. Sodium nitrate in solution and its formulation were highly stable under all stress condition i.e. acid hydrolysis, alkaline hydrolysis, thermal hydrolysis, oxidation, and photo-degradation for 24 h. The percent drug remaining in sodium nitrate solution and its formulation after 24 hours forced degradation test are shown in Figure 2.
Discussion
Chemical stability of pharmaceutical molecules was an important factor affected the safety and efficacy of drug products. Stability testing data use for understanding the quality of drug substance and drug products alter with time under the effect of the various environmental issue. The stability data helps in selecting proper formulation and package. Furthermore, it provided suitable storage condition and shelf life of drug products. Forced degradation was a process involve degradation of drug substances and drug products at condition more severe than accelerated condition \(^8\). This work studied the forced degradation of sodium nitrate solution and its formulation, sodium nitrate mouthwash for smoking cessation. Forced degradation was studied in five topics i.e. acid hydrolysis, alkaline hydrolysis, thermal hydrolysis, oxidation, and photo-degradation. Sodium nitrate mouthwash contained pharmaceutical excipients including a flavoring agent, a coloring agent, preservative, and solvent that differ from sodium nitrate solution. Thus, this work could be confirmed the effect of these excipients on the stability of sodium nitrate in the formulation. Sodium nitrate was a highly stable substance under forced degradation conditions, both its solution and formulation. Under five stress conditions, sodium nitrate does not show any degradation. In addition, the pharmaceutical excipients did not decrease the stability of sodium nitrate mouthwash. The repetition of the stress studies to obtain adequate degradation with the more severe condition and longer duration time \(^9\) were required in our further work.

Conclusion
This work studied the forced degradation of sodium nitrate solution and sodium nitrate mouthwash for smoking cessation. Results indicated that sodium nitrate in solution and in its formulation were highly stable under all stress condition i.e. acid hydrolysis, alkaline hydrolysis, thermal hydrolysis, oxidation, and photo-degradation.

Figure 2. Percent drug remaining in sodium nitrate solution and its formulation after 24 hours forced degradation test (n=3).
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