

## Thermostability of Fungi, Mite, and Dander Raw Materials under Temperature Stress Conditions

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### Abstract

**Background:** While extensive data regarding the thermostability of allergenic extracts is available, similar information for the associated raw materials is limited. This experiment investigated the thermostability of various raw material types stored under two temperatures.

**Methods:** One lot of each *Alternaria alternata* (Aa), *Dermatophagoides pteronyssinus* (Dp), and dog dander (Dd) materials were stored at 30-35°C for 16 weeks (W16) and at 60-65°C for 30 days (D30). In addition, cat dander (Cd) was stored at 30-35°C for 8 weeks (W8). Materials were extracted at regular intervals. Materials stored at <0°C were used as controls (T0). Moisture, Bradford protein content, and major allergens were determined. Alt a 1, Can f 1, Der p 1, and Der p 2 were measured by ELISA and Fel d 1 was determined by RID. Percents of the parameters obtained at W16, D30, and W8 compared to T0 were calculated (W16/T0, D30/T0, and W8/T0, respectively).

**Results:** Moisture generally remained unchanged at W16/T0, D30/T0, and W8/T0. The % protein W16/T0 for Aa, Dp, and Dd were 80.0%, 95.5%, and 153.1%, respectively; the % protein D30/T0, listed in the same order, were 56.0%, 94.1%, and 133.1%. The % Alt a 1 W16/T0 and D30/T0 values were 118.5% and 51.0%, respectively. The % Can f 1 W16/T0 and D30/T0, listed in the same order, were 69.9% and 56.4%. The % Der p 1 W16/T0 and D30/T0, were 150.4% and 86.2%, respectively; the respective % Der p 2 W16/T0 and D30/T0 were 163.8% and 25.1%. The % protein and Fel d 1 W8/T0 were 93.7% and 96.2%, respectively.

**Conclusions:** Raw materials have selective thermostability profiles. Aa, Dp, and Dd samples were stable at 30-35°C for 16 weeks and Cd for 8 weeks without any obvious deterioration. These findings could have practical applications and should be investigated further.

### Introduction

The stability of raw materials used for the production of allergenic extracts has not been studied. Stability-indicator tests to assess this component have not been defined, and are limited by the reagents available for testing.

The European Medicines Agency generally requests that real-time stability programs are implemented for all products considered active pharmaceutical ingredients, including raw materials used for the production of allergenic extracts. Greer conducted a real-time raw material stability experiment from 1998 through 2009. This experiment supported the long-term storage conditions used at Greer (0°C and low moisture, as appropriate for each material type).

Subsequently, three real-time raw material stability protocols, following ICH guidelines, were initiated at Greer. Materials classified in different homologous groups were placed on stability, and will be tested annually for five years.

In addition, Greer is preparing a stability protocol to address raw materials shipping conditions. Raw materials are typically shipped via express courier, and are normally received within one to four days after shipping, depending on location. However, non-expected delays, in very unusual situations, could potentially result in materials being in transit for up to 10 days. Therefore, a stability protocol to simulate these unusual conditions is warranted.

### Objectives

- Obtain information regarding the stability of fungi, mite, and epithelia homologous groups, placed under temperature stress conditions over short periods.
- Identify proper stability indicator test/s for each material.

### Materials and Methods

#### Source Materials

- One lot of each *Alternaria alternata*, *Dermatophagoides pteronyssinus*, dog dander, and cat dander

#### Thermal Stress Protocol

- **Ambient Relative Humidity:** 40-50%
- **Temperatures:**
  - **30-35°C**
    - *A. alternata*, *Dermatophagoides pteronyssinus*, and dog dander were stored at this temperature for 16 weeks
    - Cat dander, was included later in the study, and was stored for 8 weeks.
  - **60-65°C**
    - *A. alternata*, *Dermatophagoides pteronyssinus*, and dog dander were also stored at this temperature for 30 days.

- **Protocol Design:**
  - Raw materials stored at <0°C during the entire study period were used as controls (Time 0).
  - The testing intervals at each temperature are summarized on Table I.

Table I: Testing intervals for the raw materials stored at 30-35°C and 60-65°C

Temperature	
30-35°C (weeks)	60-65°C (days)
0	0
2	1
4	2
8	4
16	8
	16
	30

#### Source Materials Extraction and Testing

- For the purpose of consistency, all materials were extracted and tested simultaneously by the same individual at the end of the study period.
- Half a gram of each source material was extracted 1:20 (w/v) in Coca's solution.
- The extractions were performed at 2-8°C overnight under constant agitation. Extracts were clarified by centrifugation at 2,500 rpm for 10 minutes at room temperature followed by paper filtration under vacuum.
- The associated allergenic extracts were stored at 2-8°C until testing, which was completed within two weeks after extraction.
- The test methods used and the criteria to evaluate results are described in Table II.

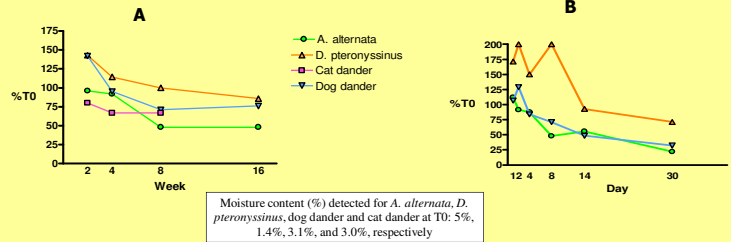
Table II: Test Methods and Specifications for the Materials

Test	Method	Raw material	Specification
Moisture Content	Gravimetric	<i>Alternaria alternata</i>	≤9.0%
		Dog dander	≤5.0%
		Cat dander	≤5.0%
		<i>Dermatophagoides pteronyssinus</i>	≤10.0%
Total Protein Content	Bradford	All extracts	50 – 200% of initial value
Major Allergens	ELISA	<i>Dermatophagoides pteronyssinus</i> (Der p 1 and Der p 2)	50 – 200% of initial value
		Dog dander (Can f 1)	50 – 200% of initial value
	RID	<i>Alternaria alternata</i> (Alt a 1)	50 – 200% of initial value
		Cat dander (Fel d 1)	50 – 200% of initial value

### Results

- All of the moisture content levels of the materials tested at the beginning of the study (Time 0) met the specifications proposed for the respective materials. The moisture contents of the materials stored at both temperatures showed a reduction associated with the length of storage (Figure 1).

Figure 1: Moisture content obtained for *A. alternata*, *D. pteronyssinus*, dog dander, and cat dander stored at 30-35°C (A) and for *A. alternata*, *D. pteronyssinus*, and dog dander stored at 60-65°C (B)



- *A. alternata*, *D. pteronyssinus*, dog dander, and cat dander were not affected during the study period when stored at 30-35°C (Figures 2A, 3A, 4A, and 5).
- *A. alternata*, *D. pteronyssinus*, and dog dander were affected when stored at 60-65°C, as determined by one or more of the parameters tested (Figures 2B, 3B, and 4B)

Figure 2: Total Protein and Major Allergen Levels in *A. alternata* Stored at 30-35°C (A) and at 60-65°C (B)

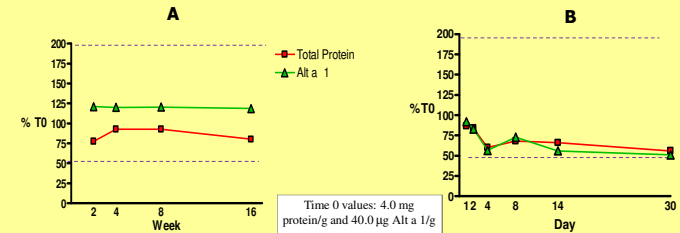


Figure 3: Total Protein and Major Allergen Levels in *D. pteronyssinus* Stored at 30-35°C (A) and at 60-65°C (B)

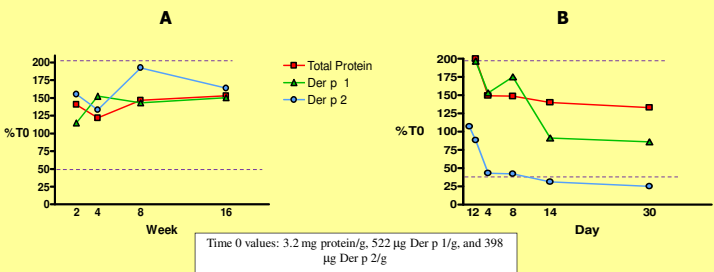


Figure 4: Total Protein and Major Allergen Levels in Dog Dander Stored at 30-35°C (A) and at 60-65°C (B)

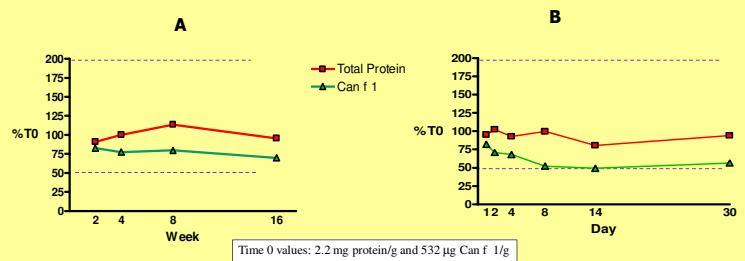
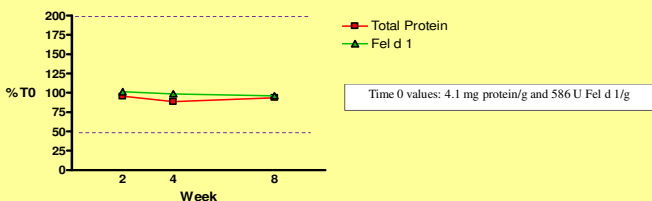


Figure 5: Total Protein and Fel d 1 contents obtained for cat dander stored at 30-35°C for 8 weeks



### Conclusions

- Raw materials have selective thermostability profiles. *Alternaria alternata*, *Dermatophagoides pteronyssinus*, and dog dander samples were stable at 30-35°C for 16 weeks and cat dander for 8 weeks without any obvious deterioration, as determined by the parameters tested. These findings could have practical applications and should be investigated further.
- The results obtained for the materials stored at 60-65°C verified the suitability of the parameters measured as stability indicator tests. These observations should be confirmed with other raw materials.
- Additional testing, including relative potency measurements, should be performed.