

Temperature Stability and Mixing Compatibility of Phenolated, Glycerinated Short Ragweed Extracts

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Abstract

Introduction: Glycerinated short ragweed extracts are incorporated into immunotherapy regimens with increasing frequency due to their improved stability compared to aqueous products. The inclusion of phenol as an anti-bacterial preservative in these products is believed by some clinicians to reduce ragweed (and other) allergen activities. However, little data are available to support these claims in ragweed extracts alone or in extract mixtures typically used for immunotherapy.

Methods: In this study, the stabilities and compatibilities of phenolated (0.2%), glycerinated (50%) short ragweed extracts were assessed after storage at moderate temperatures (20-25°C, 35-40°C) or after mixing with a variety of high-protease (fungal, insect) or low-protease (grass pollen, cat hair, dust mite) allergens. Test samples and controls contained equivalent phenol and glycerin levels, and were analyzed by short ragweed-specific Amb a 1 radial immunodiffusion assays using the identical reagents distributed by FDA for ragweed extract standardization.

Results: Short ragweed extracts at various concentrations or mixtures of short ragweed and low-protease or insect products exhibited no major changes in Amb a 1 concentrations after storage at ambient temperatures (20-25°C) for up to 12 months. Short ragweed extracts mixed with fungal products and stored under the same conditions resulted in Amb a 1 recoveries ranging from 57% (*Penicillium*) to 82% (*Aspergillus*). Exposure of short ragweed extract concentrates to higher temperatures (30-40°C) for up to 3 months reduced Amb a 1 levels by approximately 10% per month. The high levels of Amb a 1 stability observed across 11 different short ragweed solutions examined in this study demonstrated that phenol exerted very little (if any) degradative effects on this major allergen.

Conclusions: Phenolated, glycerinated short ragweed extracts retained high levels of Amb a 1 activity after storage at moderate temperatures or mixing with other extract concentrates, conditions known to compromise aqueous short ragweed allergens or mixtures. Only exposure to elevated temperature (40°C) or combination with certain fungal extracts produced noticeable changes in Amb a 1 levels. All recoveries exceeded 50% of initial Amb a 1 values, supporting the use of these solutions in a wide variety of immunotherapy procedures.

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Introduction/Overview

Phenol (hydroxybenzene) has been used for many years as an anti-microbial agent in allergenic extracts (at 0.2-0.4% w/v) and diluting fluids (at 0.4% w/v).

In closed-vial extract and diluent solutions (pH 6-8), phenol carries very little (if any) chemical reactivity. Under normal aseptic use and refrigerated storage, these solutions should remain essentially free of phenol-induced reactions. Polyphenols produced by oxidation can react directly with proteins and cause physical and chemical modifications, such as aggregation. Oxidation of phenol in closed vials is limited but can be increased by enzymes present in some extracts (browning).

Phenol-protein reactions can be reversed or inhibited by dilution or the presence of hydrophilic substances such as salts or glycerin. The effects of phenol on aqueous and glycerinated short ragweed extracts are examined here.

Materials and Methods

A freeze-dried short ragweed extract was used to assess the effects of variable phenol and glycerin concentrations on extract potency and thermal stability. Phenol was added from an 89% w/v stock solution to yield final concentrations ranging from 0.0-0.8%, and the resulting solutions were incubated at 2-8°C or 35°C for up to 2 months.

Glycerinated Greer short ragweed extracts at 2.5-320 U (μ g) Amb a 1 per mL were incubated at 20-25°C for up to 12 months and at 35-40°C for up to 3 months.

Two-component extract mixtures and single-component controls (10 mL) were formulated using glycerinated Greer extract concentrates, with each individual extract present at one-half of concentrate levels. All mixtures and controls were analyzed after storage at 20-25°C for up to 12 months.

Amb a 1 radial immunodiffusion assays were conducted using the FDA assay procedure and current lots of allergen standards and sheep anti-Amb a 1 serum.

IgE immunoblots were performed by a standard procedure utilizing 12% polyacrylamide SDS-PAGE gels (Bio-Rad), bidirectional diffusion transfers to Immobilon-PSQ PVDF membranes (Millipore), and 2 ragweed-positive human sera.

Phenolated Aqueous Extracts

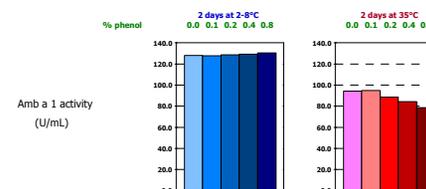
The immediate effects of phenol exposure on the activity and stability of Amb a 1 in short ragweed extracts were assessed using a freeze-dried product reconstituted with phosphate-buffered saline (PBS) in the absence of glycerin.

Phenol was added to achieve final concentrations of 0.0%, 0.1%, 0.2%, 0.4% and 0.8% w/v, and the resulting solutions were analyzed for Amb a 1 activities by RID assay during storage for up to 2 months at 2-8°C or 35°C.

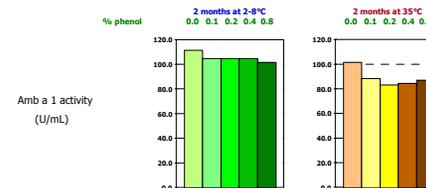
The presence of phenol levels as high as 0.8% (2X the levels contained in standardized aqueous Greer short ragweed extracts) resulted in no changes in Amb a 1 activity when stored for 2 days at 2-8°C.

Storage for 2 days at 35°C reduced Amb a 1 levels in the absence of phenol, with slightly higher reductions at 0.2-0.8% phenol concentrations (16% maximum) that remained within the established equivalence range of the Amb a 1 RID assay (\pm 20%).

Extract recoveries at 0.4% phenol were 101% and 90% after 2 days at 2-8°C and 35°C, respectively, demonstrating the favorable stability of short ragweed extracts to the phenol concentration typically used in commercial aqueous extracts and diluting fluids.



Addition of glycerin to these solutions at a 50% final concentration resulted in Amb a 1 potencies that fell within the current limits at phenol levels as high as 0.8% after 2 months storage at these temperatures.



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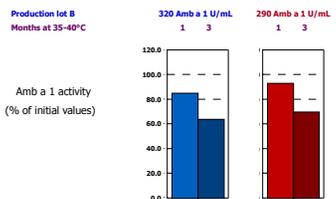
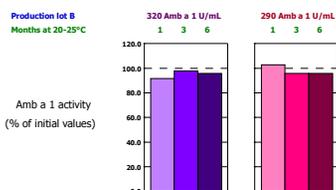
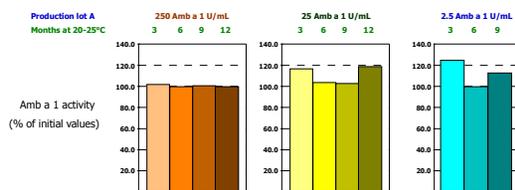


Phenolated Glycerinated Extracts

Glycerinated short ragweed extract solutions containing 0.2% phenol were examined for Amb a 1 potencies after storage for extended time periods at ambient temperatures (20-25°C) and elevated temperatures (35-40°C).

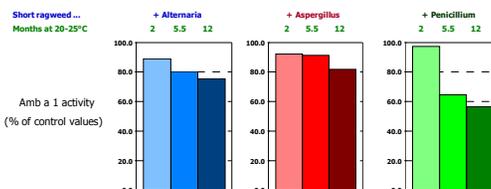
Ten extract solutions ranging from 2.5-320 Amb a 1 U/mL were prepared from four production lots of standardized glycerinated Greer short ragweed extract and analyzed during storage for up to 12 months at 20-25°C or up to 3 months at 35-40°C.

Amb a 1 recoveries for these extracts (relative to the same products stored continuously at 2-8°C) were 81-125% at 20-25°C and 64-104% at 35-40°C. Results for representative products are illustrated below.

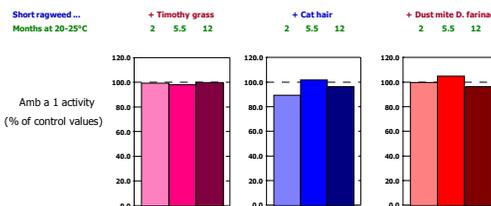
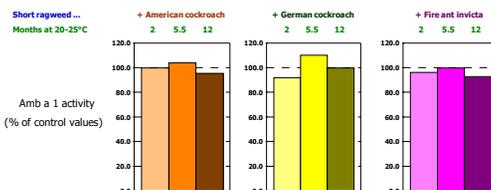


Mixing Compatibilities

Glycerinated short ragweed extracts mixed with protease-rich glycerinated fungal extracts (Alternaria, Aspergillus, Penicillium) retained 57-82% of Amb a 1 activities (compared to short ragweed-only control samples) when stored for up to 12 months at 20-25°C. Potency reductions were progressive over time with all three fungal extracts, with Penicillium extracts contributing the highest level of Amb a 1 degradation at the 5.5 month and 12 month time points. Aspergillus extracts produced the highest recoveries of Amb a 1 activity among the three fungal products.

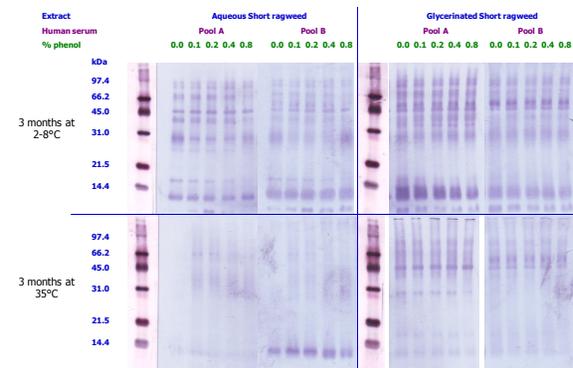


Glycerinated short ragweed extracts mixed with either protease-rich glycerinated whole-body insect extracts (American cockroach, German cockroach, fire ant invicta) or low-protease glycerinated standardized extracts (Timothy grass, cat hair, dust mite D. farinae) maintained very high levels of Amb a 1 activities (range: 90-110%) after storage for up to 12 months at 20-25°C.



IgE Immunoblot Results

Immunoblotting was performed on aqueous and glycerinated short ragweed extracts containing variable phenol concentrations to assess the IgE-binding activities of other short ragweed allergens in addition to Amb a 1. Blot membranes probed with 2 serum pools (A, B) from ragweed-sensitive patients demonstrated that phenol did not reduce or destroy IgE binding to any allergen recognized by these sera for extracts stored for 3 months at 2-8° or 35°C.



Conclusions

Phenol (0.1-0.8%) had minimal or no impact on Amb a 1 activities in aqueous short ragweed extracts stored for up to 2 days at 2-8°C or 35°C, and in glycerinated short ragweed extracts stored for 2 months at 2-8°C or 35°C. Amb a 1 levels in glycerinated products were stable with 0.2% phenol up to 12 months at 20-25°C or up to 3 months at 35-40°C.

IgE immunoblot results with 2 human serum pools showed that multiple short ragweed allergens were not affected by 0.1-0.8% phenol up to 3 months at 2-8°C or 35°C.

Phenolated, glycerinated short ragweed extracts also exhibited favorable mixing compatibilities with most high-protease and all low-protease extracts evaluated in this study. All extracts and mixtures retained at least 50% of their initial Amb a 1 activities. Fungal extracts produced the largest reductions in Amb a 1 activities.