

Mixing Compatibility of Phenolated, Glycerinated Allergenic Extracts Stored at Refrigeration or Ambient Temperatures

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Abstract

Introduction

Glycerinated allergenic extracts are utilized by allergy clinics with increasing frequency because of their enhanced physical and biochemical stabilities relative to aqueous products. Phenolated extract mixtures containing 50% glycerin are often employed as stock concentrates for testing and treatment, or as patient-specific formulations. For many common extract mixtures, the stabilities of allergens present in these solutions during typical storage or daily-use conditions have not been investigated.

Methods

The compatibilities of various phenolated (0.2%), glycerinated (50%) extracts were determined after mixing with high-protease (fungal, insect) and/or low-protease (pollen, animal, dust mite) glycerinated products and storage for up to 12 months at refrigeration (2-8°C) or ambient (20-25°C) temperatures. Test mixtures and single-extract controls were analyzed by quantitative human IgE ELISA inhibition and radial immunodiffusion assays, the methods established by FDA for extract standardization in the United States.

Results

Glycerinated dust mite, cat and dog extracts displayed near-complete recoveries of allergenic activities (70-130% of controls) after mixing with all other glycerinated extracts examined in this study during storage for up to 12 months at either 2-8°C or 20-25°C. Glycerinated short ragweed pollen extracts were highly compatible with all products tested except Penicillium at 20-25°C (57-65% recovery after 6-12 months). Glycerinated grass pollen extracts (Timothy, meadow fescue) were destabilized by mixing with insect (German cockroach) and several fungal extracts (Penicillium, Aspergillus, Cladosporium) at 2-8°C (32-57% recovery after 6-12 months), but were compatible with other fungal, pollen and dust mite extracts under these conditions.

Conclusions

Grass pollen allergens were degraded by fungal and insect extract proteases in 50% glycerin solutions stored at refrigeration and ambient temperatures. Separation of grass extracts from these (and other) high-protease products stabilized grass allergen potencies in glycerinated extract mixtures. All other glycerinated extracts tested retained moderate to high levels of allergenic activity after mixing with high-protease or low-protease glycerinated products and storage for up to 12 months at 2-8°C or 20-25°C.

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Materials and Methods

Allergen mixtures and control samples were prepared using licensed glycerinated (50% v:v) Greer extract concentrates and AP dog hair-dander extract obtained from Hollister-Stier.

100,000 BAU/mL	Timothy grass (Tim), Meadow fescue grass (Mf)
10,000 BAU/mL	Cat hair (Cat)
10,000 AU/mL	D. farinae (Df), D. pteronyssinus (Dp)
1:20-1:40 w/v	Short ragweed (SR), Giant ragweed (GR), Eastern cottonwood (Cott), White oak (Oak), Alternaria alternata (Alt), Aspergillus fumigatus (Asp), Penicillium notatum (Pen), Epicoccum nigrum (Epi), Cladosporium herbarum/C. sphaerospermum (Cla), Aureobasidium pullulans (Aur), Fusarium mix (Fus), Bipolaris/Helminthosporium (Bip), Mucor mix (Muc), Dog epithelia (Doge), American cockroach (Amco), German cockroach (Geco), Fire ant invicta (FA)
1:100 w/v	Hollister-Stier AP Dog hair-dander (Dogd)

Two-component extract mixtures and single-component extract controls were formulated at one-tenth of concentrate strengths in 2 mL or 5 mL total volumes using 50% glycerol-normal saline diluent. All samples were analyzed after storage for up to 12 months at 2-8°C or 20-25°C.

IgE ELISA inhibition assays were performed to determine the allergenic potencies of grass pollen, tree pollen, dust mite, dog and Alternaria extracts. Parallel dose-response curves bracketing 50% inhibition levels were observed for reference (controls) and test antigens (mixtures) with IgE-positive human serum pools for each of the target allergens. Relative IgE-binding potencies were calculated using a parallel line bioassay spreadsheet similar to those employed for lot release and stability testing of standardized grass pollen and dust mite extracts.

Radial immunodiffusion assays for short ragweed allergen Amb a 1 and cat hair allergen Fel d 1 employed specific allergen standards, anti-Amb a 1 and anti-Fel d 1 sheep antisera, and incubation conditions identical to those used for standardization of short ragweed and cat hair extracts.

Two-site ELISA analysis of Alternaria allergen Alt a 1 utilized rabbit anti-Alt a 1 capture and biotinylated probe antibodies, with results obtained by log-linear regression of calibrated allergen standards and multiple test sample dilutions.

Percent recoveries for extract mixtures were expressed relative to the activities of single-extract controls containing identical concentrations of test allergens. Recoveries were typically within 10% of mean values for replicate samples.

Results were assessed for significance by T test ($p \leq 0.05$).

Mixing Compatibilities at 2-8°C

Extract activities in mixtures were determined after storage for 1-12 months at 2-8°C. Mean recoveries of 70% or higher (stable, green shading), 50-69% (risky, yellow shading) and below 50% (unstable, red shading) are illustrated below.

Target ↓	Mean % Recovery after mixing and storage for ...			
	Months at 2-8°C			
	1	3	6	12
Tim or Mf				
Alt	94	70	71	
Asp	82	77	38	49
Pen	62	66	56	32
Cla	76	102	45	
Epi	69		96	
Aur	72		177	
Fus	152		73	
Muc	70		78	
SR				
Alt		112	93	
Asp		112	95	
Pen		93	86	
Amco	126		89	
Geco	100		90	
Df or Cat				
Alt		102	96	
Asp		124	99	
Pen		113	105	
SR	107	122	115	141
Doge or Dogd				
Alt		105	105	
Asp		104	105	
Pen		95	105	
SR	96	113	91	119
Alt				
Asp		111		94
Pen		80		98
Cla		95		87
Epi		77		74
Cott or Oak				
Alt			90	
Asp			72	
Pen			85	
+				
+				
Bip	94	83	104	
Amco		101		
Geco		82	57	
FA		135		
Df			101	89
Dp			87	103
SR	104	108	92	98
GR	100	90	99	117
+				
+				
Tim	87	108	93	90
Df		100	113	88
Cat		104	117	97
Doge		99	94	99
Dogd		98	97	103
+				
+				
Amco	99		111	
Geco	109		106	
GR	110	110	114	137
+				
+				
Amco			113	89
Geco			104	87
FA			112	106
GR	91	114	82	105
+				
+				
Aur		68		71
Fus		74		74
Muc		78		75
Bip		74		86
+				
+				
Amco			125	
Geco			114	
FA			105	

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Mixing Compatibilities at 20-25°C

Extract recoveries in mixtures stored for 1-12 months at 20-25°C are tabulated below, using the same ranges and colors as the 2-8°C stability data. In several combinations, ragweed extracts stabilized grass and dog allergens, as indicated by the elevated target extract recoveries (above 130%) for these mixtures relative to grass-only and dog-only extract control samples.

Target ↓	Mean % Recovery after mixing and storage for ... Months at 20-25°C				+	Mean % Recovery after mixing and storage for ... Months at 20-25°C				
	1	3	6	12		1	3	6	12	
Tim or Mf	Alt	41	34	11	Amco	40	32			
	Asp	27	19		Geco	31	25			
	Pen	18	9		FA	48	54			
	Cla	70	80		SR	86	82	80	127	
	Bip	63	34		GR	80	83	107	175	
SR	Alt		89	87	75	Tim	100	98	100	97
	Asp		92	92	82	Df		102	101	93
	Pen		98	65	57	Cat		101	117	96
	Amco		100	104	95	Doge		95	99	99
	Geco		92	110	100	Dogd		104	104	99
Df or Cat	SR	94	129	121	118	GR	79	110	117	115
	Asp		105	146	121	219	GR	101	170	112
Doge or Dogd	SR	105	146	121	219	GR	101	170	112	198
	Alt			98		Tim			81	

Statistical Significance

Target extract recoveries for each mixture were analyzed by T tests assuming equal variances, with statistical significance achieved at p values at or below 0.05.

Most combinations shaded in yellow and all mixtures highlighted in red were found to exhibit significant levels of allergen degradation.

Compatibility Charts: 2-8°C

Based on the results from these and other recent Greer R&D studies, the expected mixing compatibilities of glycerinated extracts stored at 2-8°C are summarized using the following charts, with specific allergen combinations noted as compatible (green), risky (yellow) or compromised (red).

Stability of ... ↓	After mixing and storage for 1-3 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Green	Green	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓	After mixing and storage for 6 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Yellow	Yellow	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓	After mixing and storage for 12 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Compatibility Charts: 20-25°C

The mixing compatibilities of glycerinated extracts stored at 20-25°C are estimated below, using the same risk categories and colors displayed on the 2-8°C compatibility charts. Grass allergens were degraded by fungal or insect extracts after short (1 month) exposures at 20-25°C.

Stability of ... ↓	After mixing and storage for 1-3 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓	After mixing and storage for 6 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Yellow	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓	After mixing and storage for 12 months with ...				
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Yellow	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Conclusions

Glycerinated grass pollen allergens were degraded significantly after mixing with several glycerinated fungal or insect extracts and storage for 6-12 months at 2-8°C or for 1-6 months at 20-25°C.

Short ragweed allergen Amb a 1 was destabilized by mixing with Penicillium after 6-12 months at 20-25°C, but retained high levels of activity after mixing and storage with many other high-protease extracts.

All other mixtures of high-protease and low-protease extracts examined in this study were compatible after storage for up to 12 months at 2-8°C or 20-25°C.

These data support the current clinical utilization of glycerinated allergen mixtures for diverse testing and injection treatment regimens, as well as investigations of non-injection modes of immunotherapy, including multi-allergen sublingual-oral administration.

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Compatibility Charts: 2-8°C

Based on the results from these and other recent Greer R&D studies, the expected mixing compatibilities of glycerinated extracts stored at 2-8°C are summarized using the following charts, with specific allergen combinations noted as compatible (green), risky (yellow) and compromised (red).

Stability of ... ↓

After mixing and storage for 1 month with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Green	Green	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 3 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Green	Green	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 6 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Yellow	Yellow	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 12 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Compatibility Charts: 20-25°C

The mixing compatibilities of glycerinated extracts stored at 20-25°C are estimated below, using the same risk categories and colors displayed on the 2-8°C compatibility charts. Grass allergens were degraded by fungal or insect extracts after short (1 month) exposures at 20-25°C.

Stability of ... ↓

After mixing and storage for 1 month with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 3 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Green	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 6 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Yellow	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green

Stability of ... ↓

After mixing and storage for 12 months with ...

	High-protease		Low-protease		
	Fungi	Insects	Ragweeds	Dust mites	Cat/Dog
Grass pollen	Red	Red	Green	Green	Green
Ragweed pollen	Yellow	Green	Green	Green	Green
Other pollen	Green	Green	Green	Green	Green
Dust mites	Green	Green	Green	Green	Green
Cat/Dog	Green	Green	Green	Green	Green
Fungi	Green	Green	Green	Green	Green