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

JG Grier, PhD, DM LeFevre, BS, EA Duncan, BS, KN Whitaker, BS, RE Esch, PhD and TC Coyne, MD
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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 and AP dog hair-dander extract obtained from Hollister-Stier. Below 50% (unstable, red shading) are illustrated below.

Materials and Methods
Mixing Compatibilities at 2-8°C

Glycerinated allergenic 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 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.

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% (risks, yellow shading) and below 50% (unstable, red shading) are illustrated below.

<table>
<thead>
<tr>
<th>Target</th>
<th>Mean % Recovery after mixing and storage for ...</th>
<th>Mean % Recovery after mixing and storage for ...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Months at 2-8°C</td>
<td>Months at 2-8°C</td>
</tr>
<tr>
<td></td>
<td>+ 1 3 6 12</td>
<td>+ 1 3 6 12</td>
</tr>
<tr>
<td>Alt</td>
<td>+ 94 76 71</td>
<td>+ 94 76 104</td>
</tr>
<tr>
<td>Asp</td>
<td>+ 94 76 71</td>
<td>+ 94 76 104</td>
</tr>
<tr>
<td>Pen</td>
<td>+ 62 66 96</td>
<td>+ 62 97 57</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 76 90 99</td>
<td>+ 76 90 99</td>
</tr>
<tr>
<td>Epi</td>
<td>+ 69 90 100</td>
<td>+ 72 117 137</td>
</tr>
<tr>
<td>Fus</td>
<td>+ 152 73 90</td>
<td>+ 109 92 117</td>
</tr>
<tr>
<td>Muc</td>
<td>+ 70 78 100</td>
<td>+ 105 99 117</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 112 91 93</td>
<td>+ 108 93 90</td>
</tr>
<tr>
<td>Asp</td>
<td>+ 112 95 99</td>
<td>+ 100 93 91</td>
</tr>
<tr>
<td>Pen</td>
<td>+ 93 86 91</td>
<td>+ 104 93 97</td>
</tr>
<tr>
<td>SR</td>
<td>+ 126 89 99</td>
<td>+ 104 94 99</td>
</tr>
<tr>
<td>Amco</td>
<td>+ 100 90 90</td>
<td>+ 89 102 101</td>
</tr>
<tr>
<td>Geco</td>
<td>+ 107 115 143</td>
<td>+ 100 106 106</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 102 96 99</td>
<td>+ 109 94 99</td>
</tr>
<tr>
<td>Asp</td>
<td>+ 126 99 99</td>
<td>+ 113 95 105</td>
</tr>
<tr>
<td>Pen</td>
<td>+ 113 105 111</td>
<td>+ 116 110 117</td>
</tr>
<tr>
<td>SR</td>
<td>+ 107 115 143</td>
<td>+ 113 114 142</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 105 105 105</td>
<td>+ 113 105 105</td>
</tr>
<tr>
<td>Asp</td>
<td>+ 104 105 105</td>
<td>+ 104 105 105</td>
</tr>
<tr>
<td>SR</td>
<td>+ 112 114 118</td>
<td>+ 112 114 118</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 95 99 99</td>
<td>+ 112 116 118</td>
</tr>
<tr>
<td>Dmg</td>
<td>+ 91 98 99</td>
<td>+ 95 99 99</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 111 95 95</td>
<td>+ 111 95 95</td>
</tr>
<tr>
<td>Cott</td>
<td>+ 80 87 87</td>
<td>+ 68 71</td>
</tr>
<tr>
<td>Oak</td>
<td>+ 77 77 77</td>
<td>+ 74 74</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 111 95 95</td>
<td>+ 111 95 95</td>
</tr>
<tr>
<td>Cott</td>
<td>+ 90 90 90</td>
<td>+ 83 83</td>
</tr>
<tr>
<td>Oak</td>
<td>+ 72 72 72</td>
<td>+ 78 78</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 85 85 85</td>
<td>+ 74 74</td>
</tr>
<tr>
<td>Cott</td>
<td>+ 90 90 90</td>
<td>+ 125 125</td>
</tr>
<tr>
<td>Oak</td>
<td>+ 83 83 83</td>
<td>+ 74 74</td>
</tr>
<tr>
<td>Bip</td>
<td>+ 72 72 72</td>
<td>+ 114 114</td>
</tr>
<tr>
<td>Cott</td>
<td>+ 85 85 85</td>
<td>+ 105 105</td>
</tr>
</tbody>
</table>
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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.

<table>
<thead>
<tr>
<th>Target</th>
<th>Mean % Recovery after mixing and storage for 1-12 months at 20-25°C</th>
<th>Months at 20-25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1 3 6 12</td>
<td>+ 1 3 6 12</td>
</tr>
<tr>
<td>Alt</td>
<td>45 21 11</td>
<td>Amco 40 12</td>
</tr>
<tr>
<td>Asp</td>
<td>27 11</td>
<td>Geco 31 10</td>
</tr>
<tr>
<td>Tim or MF</td>
<td>18 5</td>
<td>FA 48 94</td>
</tr>
<tr>
<td>Pon</td>
<td>70 80</td>
<td>SR 86 82 80 117</td>
</tr>
<tr>
<td>Cia</td>
<td>63 54</td>
<td>GR 80 83 107 175</td>
</tr>
<tr>
<td>Bip</td>
<td>1 3 6 12</td>
<td>+ 1 3 6 12</td>
</tr>
<tr>
<td>+</td>
<td>85 77 75</td>
<td>Tim 100 98 100 97</td>
</tr>
<tr>
<td>Alt</td>
<td>91 92 92</td>
<td>Asp 102 101 91</td>
</tr>
<tr>
<td>Asp</td>
<td>98 65 57</td>
<td>Pen 101 117 95</td>
</tr>
<tr>
<td>SR</td>
<td>110 104 85</td>
<td>Doge 95 99 99</td>
</tr>
<tr>
<td>Amco</td>
<td>92 110 80</td>
<td>Dogd 104 104 99</td>
</tr>
<tr>
<td>Geco</td>
<td>97 120 96</td>
<td>FA 97 100 97</td>
</tr>
<tr>
<td>+</td>
<td>1 3 6 12</td>
<td>+ 1 3 6 12</td>
</tr>
<tr>
<td>DF or Cat</td>
<td>94 120 121 116</td>
<td>GR 79 110 117 115</td>
</tr>
<tr>
<td>Doge or Dogd</td>
<td>105 146 121 220</td>
<td>Tim 101 170 112 128</td>
</tr>
<tr>
<td>Alt</td>
<td>98 98 98</td>
<td>Tim 81 98 98 98</td>
</tr>
</tbody>
</table>

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).

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.

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

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#### Stability of ... After mixing and storage for 1 month with ... 20-25°C

<table>
<thead>
<tr>
<th>Stability of ...</th>
<th>After mixing and storage for 1 month with ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>High-protease</td>
</tr>
<tr>
<td>Grass pollen</td>
<td></td>
</tr>
<tr>
<td>Ragweed pollen</td>
<td></td>
</tr>
<tr>
<td>Other pollen</td>
<td></td>
</tr>
<tr>
<td>Dust mites</td>
<td></td>
</tr>
<tr>
<td>Cat/Dog</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
</tbody>
</table>

#### Stability of ... After mixing and storage for 3 months with ... 20-25°C

<table>
<thead>
<tr>
<th>Stability of ...</th>
<th>After mixing and storage for 3 months with ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>High-protease</td>
</tr>
<tr>
<td>Grass pollen</td>
<td></td>
</tr>
<tr>
<td>Ragweed pollen</td>
<td></td>
</tr>
<tr>
<td>Other pollen</td>
<td></td>
</tr>
<tr>
<td>Dust mites</td>
<td></td>
</tr>
<tr>
<td>Cat/Dog</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
</tbody>
</table>

#### Stability of ... After mixing and storage for 6 months with ... 20-25°C

<table>
<thead>
<tr>
<th>Stability of ...</th>
<th>After mixing and storage for 6 months with ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>High-protease</td>
</tr>
<tr>
<td>Grass pollen</td>
<td></td>
</tr>
<tr>
<td>Ragweed pollen</td>
<td></td>
</tr>
<tr>
<td>Other pollen</td>
<td></td>
</tr>
<tr>
<td>Dust mites</td>
<td></td>
</tr>
<tr>
<td>Cat/Dog</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
</tbody>
</table>

#### Stability of ... After mixing and storage for 12 months with ... 20-25°C

<table>
<thead>
<tr>
<th>Stability of ...</th>
<th>After mixing and storage for 12 months with ...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>High-protease</td>
</tr>
<tr>
<td>Grass pollen</td>
<td></td>
</tr>
<tr>
<td>Ragweed pollen</td>
<td></td>
</tr>
<tr>
<td>Other pollen</td>
<td></td>
</tr>
<tr>
<td>Dust mites</td>
<td></td>
</tr>
<tr>
<td>Cat/Dog</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
</tbody>
</table>

### 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.