# The Importance of Tablet Formulation on Allergen Release Kinetics and Efficiency: Comparison of Freeze-dried and Compressed Grass Pollen Sublingual Allergy Immunotherapy Tablet Formulations

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## ABSTRACT

**Purpose:** Efficient delivery of allergens to the sublingual mucosa is a prerequisite for successful sublingual immunotherapy (SLIT) for allergy, and in order to become available to immune-competent cells embedded in the sublingual mucosa, allergens need to be delivered in a soluble form. Delivery of solubilized allergens poses a particular challenge for tablet-based allergy immunotherapy, in which allergens are administered under the tongue in the form of dry tablets and need to be dissolved rapidly in a small volume of saliva, with little or no agitation. The purposes of this article were to compare the properties of 2 different pharmaceutical SLIT-tablet formulations, freeze-dried and compressed, and to examine how the tablet formulation affects the efficiency with which allergen is delivered from the dry state of the tablet into soluble form.

**Methods:** Two SLIT-tablet formulations, both indicated for grass pollen allergic rhinitis and containing grass pollen extract as the active ingredient, were examined with regard to tablet disintegration times, allergen dissolution kinetics, dependency on solvent volume and agitation, and the achieved recovery of the grass allergen content in soluble form with each tablet.

**Findings:** The freeze-dried and the compressed SLIT-tablet formulations differed markedly with respect to efficiency of allergen release. The freeze-dried tablet disintegrated faster and released grass allergen into solution with a release rate higher than that of the compressed formulation and, in contrast to the compressed formulation, achieved full recovery of the allergen content in soluble form in a small volume of solvent.

**Implications:** Rapid and complete release of soluble allergen in a small volume of solvent, as demonstrated by the freeze-dried formulation, are key elements of efficient sublingual allergen delivery by SLIT-tablets. Complete allergen release means that the full allergen dose of the tablet is recovered from the tablet and made available to the sublingual immune system in soluble form, and rapid release ensures that the immune system becomes exposed to the highest possible dose of soluble allergen for the maximal duration before swallowing. In contrast, a SLIT-tablet formulation that provides incomplete and slower allergen release will likely require a higher allergen content compared to the more efficient formulation, in order to achieve the same dose of soluble allergen, consequently leading to an excess load of allergen that becomes swallowed without having been made immunologically available. (*Clin Ther*. 2019;41:742–753) © 2019 Published by Elsevier Inc.

**Key words:** allergen delivery, allergen recovery, SLIT-tablet formulation, sublingual immunotherapy, sublingual tablet disintegration.

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## INTRODUCTION

In recent years, a substantial number of large-scale clinical trials have provided evidence that establishes tablet-based allergy sublingual immunotherapy (SLIT) as a clinically effective and well-tolerated treatment option for a growing number of important respiratory allergens.[1–8] The most common indication for SLIT-tablet treatment is allergic rhinitis in adults and children,[9–12] and in one instance is a SLIT-tablet indicated for house dust mite (HDM) allergic asthma.[8] In addition to SLIT-tablets, SLIT products also include aqueous formulations of allergen extracts, SLIT-drops. All approved SLIT-tablets available today are based on natural allergen extracts as the active ingredient. Allergen extracts are complex mixtures of major and minor allergens and other nonallergenic substances specific for the source material, and it is the combined activity of these components that is involved in the immunologic and clinical effects of allergy immunotherapy (AIT).

The first and most important challenge for the development of pharmaceutical-grade allergen extract–based AIT products was to develop production and quality-control procedures that would ensure sufficient consistency in the batch-to-batch allergenic potency of the allergen extracts. In Europe, this challenge was met by the individual manufacturers, who developed in-house standards and procedures to maintain consistent batch-to-batch potencies.[13–17] In the United States, allergen extract standardization is also done by the manufacturers, but with a common set of allergen standards issued by the US authorities,[18] and in Japan, standardization of new AIT products is the responsibility of the Japanese Society of Allergology (JSA), who, based on JSA-developed standards and procedures, assigns the potency to individual products.[19]

Although allergen extracts from different producers differ with regard to allergen composition, which hampers direct comparison between extracts, the standardization has now in general reached a level at which only high-quality and consistently produced allergen extracts are used for the manufacture of registered AIT products. However, when the development of AIT SLIT-tablets was initiated, new challenges emerged: how to formulate allergen extracts in a tablet formulation that is compatible with allergen extract biochemistry, the anatomy and immunologic structures of the sublingual mucosa, as well as with the patient's desire for convenience. The downstream immunologic mechanisms of SLIT have not yet been completely elucidated, but it is agreed that following sublingual administration allergens become internalized by local immune-competent cells resident in the sublingual mucosa, such as antigen-presenting oral Langerhans cells.[20–22] This mechanism requires allergens to be available on the sublingual mucosal surface in solubilized form in order to be captured by the antigen-presenting cells. For the aqueous SLIT-drops, soluble allergens are readily available, initially in a concentration that corresponds to the concentration of allergen in the product. For SLIT-tablets, however, the allergens need to be released from the tablet and solubilized in saliva before they become available to the mucosal immune system. Release and solubilization of the allergen content of SLIT-tablets in a small volume of saliva obviously poses a significant challenge, and since allergen release must be completed within a short period of time specified by the manufacturer, the specifications and performance of the tablet formulation become even more important. With regard to the freeze-dried tablet, it is recommended that swallowing be avoided for about 1 minute after placement of the tablet under the tongue.[23,24] With regard to the compressed tablet, the recommendation is to place the tablet under the tongue until completely dissolved (at least 1 minute) and then swallow.[25]

Currently, approved SLIT-tablets are available in 2 different formulations. One formulation is a tablet manufactured by compression of the allergen extract together with microcellulose, lactose, and other excipients,[25] and the other is a freeze-dried tablet based on gelatin and mannitol.[23] SLIT-tablets based on the compressed formulation are available for HDM and grass pollen allergy.[3,26] Freeze-dried SLIT tablets with the same tablet formulation as examined herein are available for HDM allergy and allergic asthma,[7,8] as well as grass pollen, ragweed, and Japanese cedar related allergic rhinitis[1,4] and are in development for allergy to birch and related trees.[5]

Here, the two SLIT-tablet formulations were examined and compared with regard to tablet disintegration time and other disintegration characteristics, allergen dissolution kinetics, and

allergen recovery. Both tablets contain natural, unmodified grass pollen extract as the active ingredient, and the nominal strengths are measured in proprietary units, SQ-T and IR, respectively, defined by the manufacturers. A 300 IR grass SLIT-tablet was used as a representative of the compressed SLIT-tablet and a 75,000 SQ-T grass SLIT-tablet was used as a representative of the freeze-dried SLIT-tablet formulation.

## MATERIALS AND METHODS

All experiments were done *in vitro*, and all samples were processed in parallel in identical conditions unless otherwise indicated.

### Test Samples

Freeze-dried SQ grass SLIT-tablets‡ (75,000 SQ-T; lot no. 1637535, expiry date December 2020) were obtained from the manufacturer (SQ is a method for standardization of biological potency, major allergen content, and complexity of the allergen extract). Compressed IR grass SLIT-tablets§ (300 IR; lot no. 3146-1N2-1, expiry date August 2019) were obtained from a pharmacy in France. All experiments were finalized before the respective expiration dates. The compressed IR grass SLIT-tablet is available as a 100 IR tablet intended for allergen up-dosing as well as a 300 IR tablet intended as the maintenance dose. No up-titration of the freeze-dried SQ grass SLIT-tablet is needed and therefore only the maintenance dose of 75,000 SQ-T is available. Here, only the maintenance-dose tablets of 75,000 SQ-T and 300 IR, respectively, were examined.

### Assay Buffer

A 100 mmol/L phosphate buffer (pH 6.8) supplemented with 0.125% casein was used for disintegration and dissolution experiments. The composition of the assay buffer was similar to human saliva with regard to pH value, ionic strength, and total protein content.[27]

‡ Trademark: Grazax® (ALK-Abelló A/S, Hørsholm, Denmark).

§ Trademark: Oralair® (Stallergenes-Greer, London, United Kingdom).

### Tablet Disintegration Test

Tablet disintegration was done according to the Japanese pharmacopeia.[28] Briefly, tablets (n = 6) were deposited into a submersible mesh basket (1.8−2.2 mm mesh size) and agitated vertically in assay buffer at 37°C until disintegrated. Disintegration was considered complete when all of the tablet residue had passed through the mesh.

### Total Tablet Major Allergen Content

Allergens were released from the freeze-dried SQ grass SLIT-tablets by depositing tablets (n = 4) in parallel experiments into 1 mL of assay buffer followed by gentle agitation after which the tablets dissolved within seconds. Without further processing of the sample, the content of solubilized Phl p 5 was measured by enzyme-linked immunosorbent assay (ELISA).

With the compressed IR grass SLIT-tablet, only a minimal yield of solubilized grass was achieved by this method, so a more elaborate procedure was employed: In parallel experiments, the compressed IR grass SLIT-tablets (n = 4) were deposited into 10 mL of assay buffer and subjected to 3 repetitions of vortexing with 5-minute intervals. Following centrifugation (10 minutes, 11,000 rpm, room temperature) to separate undissolved tablet residue from the supernatant, the content of grass group 5 allergen in the supernatant was measured by ELISA.

### Grass Group 5 ELISA

Quantitative determination of grass group 5 major allergen was determined by ELISA as described elsewhere.[29] Briefly, a microtiter plate was coated with a mixture of 2 anti−group 5 allergen monoclonal antibodies. The plates were blocked with casein and incubated with sample for 1 hour at room temperature. After washing, sample solution was applied to the plate and incubated overnight at 5°C. After washing, biotinylated anti−grass pollen rabbit antibody (ALK-Abelló) was added, followed by peroxidase-conjugated streptavidin (Agilent Technologies, Glostrup, Denmark).

### Tablet Dissolution/Allergen Release Kinetics

A dissolution test was performed according to the paddle method of the Japanese pharmacopeia[28] using a mini-vessel (200 mL) Distek Model 2500 (Distek

Inc, North Brunswick, New Jersey). Tablets were deposited into assay buffer (100 mL, 37°C) and agitated with a paddle speed of 50 rpm. Aliquots were collected at 15, 30, 45, 60, 90, 120, 180, 300, and 600 seconds, and the amount of solubilized grass group 5 allergen was determined by ELISA.[29]

### Statistical Analysis

All statistical analyses were done using GraphPad Prism version 7.02 (GraphPad Software, San Diego, California). An unpaired *t* test with Welch correction was used for comparisons of tablet allergen content, disintegration times, and dissolution.

## RESULTS

### Tablet Disintegration Test

Tablets were deposited into assay buffer, and the time to disintegration was measured. The data in Figure 1 show the disintegration times of the freeze-dried SQ grass SLIT-tablets and the compressed IR grass SLIT-tablets. While the freeze-dried SQ grass SLIT-tablets disintegrated completely within 1 second, the compressed IR grass SLIT-tablets did not disintegrate to a degree at which remaining particle size was less than the pharmacopeia-specified 1.8 to 2.2 mm until after 31 (1) seconds in solution (mean [SD]; n = 6). The difference between the disintegration times of the 2 tablets was significant ($P < 0.001$). In contrast to the freeze-dried SQ grass SLIT-tablets, a substantial amount of solid tablet residue was visible after the compressed IR grass SLIT-tablets had disintegrated and was readily recovered as a particulate precipitate following centrifugation (Figure 2).

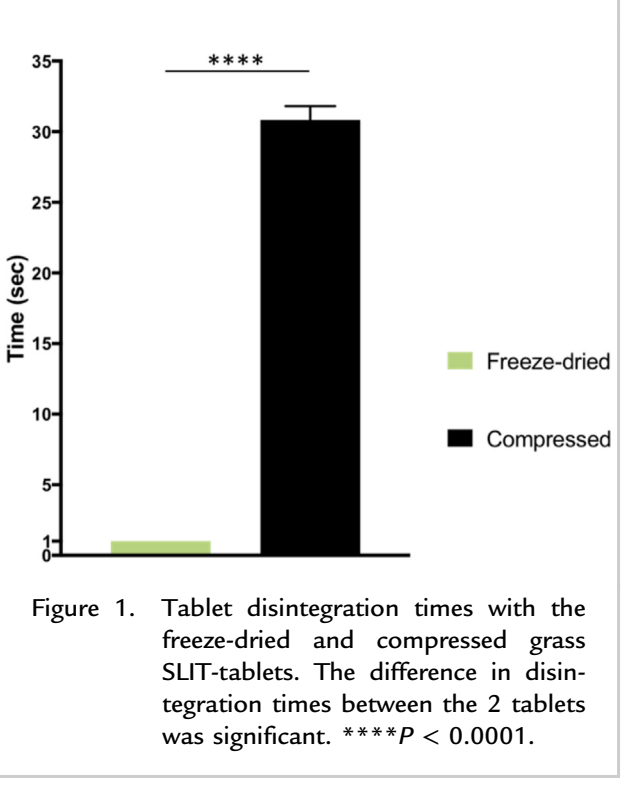


Figure 1. Tablet disintegration times with the freeze-dried and compressed grass SLIT-tablets. The difference in disintegration times between the 2 tablets was significant. ****$P < 0.0001$.

### Grass SLIT-Tablet Allergen Content and Recovery

The allergen content of the freeze-dried SQ grass pollen SLIT-tablets was retrieved by dissolving the tablets in 1 mL of assay buffer with gentle agitation, and the amount of Phl p 5 that was released from the tablet in soluble form was subsequently quantified by ELISA. In the ELISA assay, the freeze-dried 75,000 SQ-T grass SLIT-tablet in-house reference extract, in which 75,000 SQ-T corresponds to 15 µg of Phl p 5, was used as a standard. The amount of Phl p 5 recovered from the freeze-dried SQ grass SLIT-tablet was determined to be 74,675 (1623) SQ-T (mean [SD]; n = 4) (data not shown), corresponding to 14.9 (0.3) µg of Phl p 5 (Table).

A different method of recovering the full amount of allergen from the compressed IR grass SLIT-tablet had to be applied compared to the method used for the freeze-dried SQ grass SLIT-tablet. Dissolution of the compressed IR grass SLIT-tablet in 1 mL of assay buffer with gentle agitation did not provide a convincing yield (data not shown), so the dissolution volume was increased, and agitation was intensified. The yield of solubilized grass group 5 allergen obtained by this method corresponded to 12.7 (0.3) µg (mean [SD]; n = 4) of grass group 5 allergen as determined by ELISA (Table). Although similar, the amounts of grass group 5 major allergen recovered from the 2 tablets were statistically significantly different ($P < 0.0001$).

The purpose of assessing the grass group 5 major allergen content of the compressed IR grass SLIT-tablet was not to make direct comparisons of the absolute allergen content between the 2 tablet types but to get an indication of the maximal amount of allergen that could be recovered from the compressed IR grass SLIT-tablet, and to obtain a reference for the release kinetics experiments.

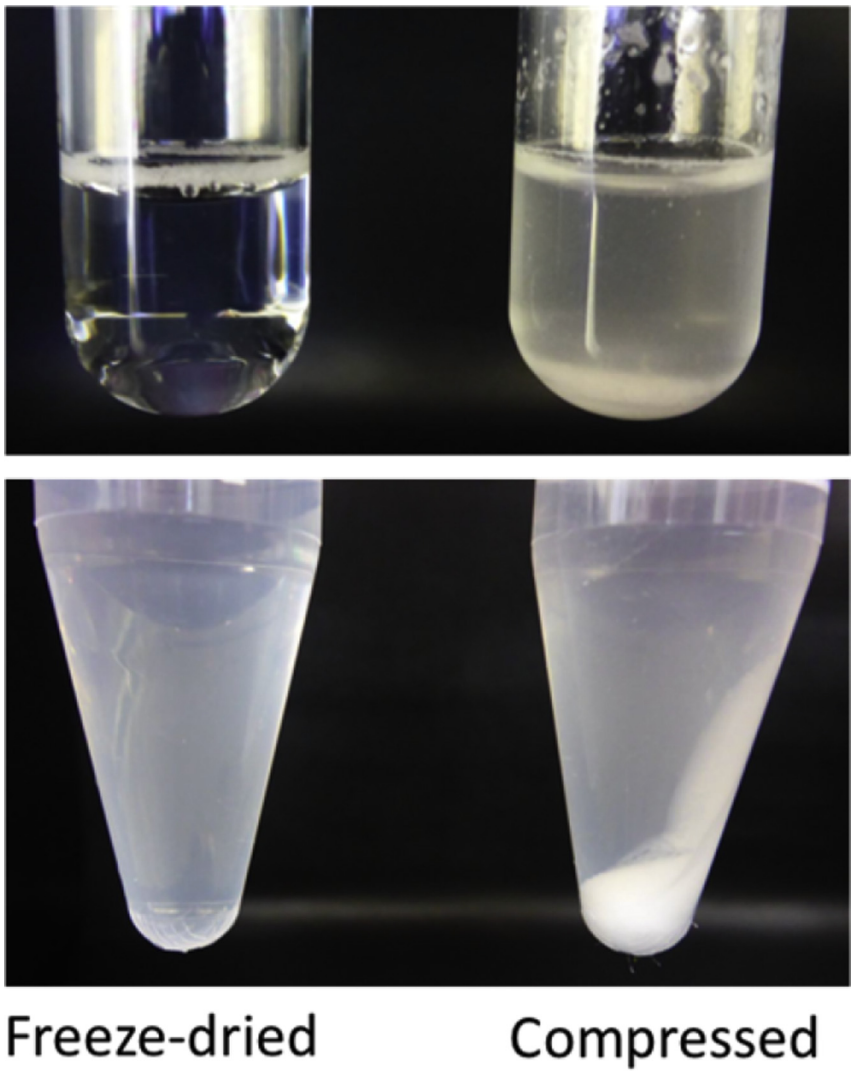


Figure 2. After dissolution of the freeze-dried SQ grass SLIT-tablet in assay buffer, the solution remained clear. In contrast, dissolution of the compressed IR grass SLIT-tablet gave rise to a turbid appearance of the solvent (upper panel). Following centrifugation, a solid precipitate was recovered from the compressed tablet, while no residue from the freeze-dried tablet was visible (lower panel).

### Kinetics of Grass Major Allergen Release

The kinetics of grass allergen release from the freeze-dried SQ and the compressed IR SLIT-tablets were measured by depositing the tablets into 100 mL of assay buffer with gentle stirring, followed by quantification of solubilized grass group 5 major allergen. Aliquots were removed after 15, 30, 45, 60, 90, 120, 180, 300, and 600 seconds, and the amounts of solubilized grass group 5 allergen were measured by ELISA. Figure 3 shows the kinetics of absolute grass group 5 allergen release from 0 to 600 seconds and 0 to 120 seconds. Allergen release from the freeze-dried SQ grass SLIT-tablet occurred rapidly and efficiently, with achieved mean release amounts of 13.1 (1.7) μg (mean [SD]; n = 4) of Phl p 5 after 15 seconds, and 14.1 (1.0) μg (mean [SD]; n = 4) of Phl p 5 after 30 seconds in solution, whereafter the cumulated allergen concentration remained stable for

Table. The total amount of grass group 5 major allergen recovered from the freeze-dried and compressed-grass SLIT tablets, measured by ELISA, using the 75,000 SQ-T in-house reference as a standard.

| Formulation | Nominal Strength | Recovered Grass Group 5 Allergen, mean (SD) (n = 4) | *P* |
|---|---|---|---|
| Freeze-dried | 75,000 SQ-T | 14.9 (0.3) | <0.0001 |
| Compressed | 300 IR | 12.7 (0.3) | |

ELISA = enzyme-linked immunosorbent assay; SLIT = sublingual immunotherapy.

the duration of the experiment. Slower and less comprehensive allergen release was achieved with the compressed IR grass SLIT-tablet, which yielded 1.1 (0.09) μg (mean [SD]; n = 4) of solubilized grass group 5 allergen after 60 seconds in solution, increasing to 2.0 (0.2) μg (mean [SD]; n = 4) after 120 seconds. The differences between the 2 tablets with regard to grass group 5 major allergen dissolution were significant ($P < 0.001$ at 15 seconds, $P < 0.0001$ at all other time points).

Figure 4 shows the allergen release kinetics of the 2 grass pollen SLIT-tablets on a relative scale in which the amounts of grass group 5 allergens from the Table were used as a measure of maximal release. On this scale, a mean of 87.9% (11.4%) of the freeze-dried SQ grass tablet Phl p 5 content had been solubilized after 15 seconds, and 94.6% (6.7%) after 30 seconds. The difference between the reference value of 14.9 μg of Phl p 5 and the amount of allergen released after 15 seconds in solution was not statistically significant, so complete allergen release, and therefore full recovery of the tablet allergen content, was achieved with the freeze-dried SQ grass SLIT-tablet already after 15 seconds. In comparison, the recovery of grass group 5 allergen from the compressed IR grass SLIT-tablet reached 8.7% (0.7%) after 60 seconds, increasing to 15.8% (1.6%) of the amount of grass group 5 shown in the Table after 120 seconds in solution. Full recovery of grass group 5 major allergen was not achieved with the compressed IR grass SLIT-tablet even after 10 minutes in solution.

## DISCUSSION

With an anatomy characterized by high permeability and the presence of highly immune-competent lymphoid structures, the sublingual mucosa is an attractive site for allergen administration with SLIT,[22,30,31] and although not completely understood, tolerogenic immunologic mechanisms of the oral mucosa may contribute positively to the immunologic and subsequent clinical effects of SLIT.[22] Furthermore, the sublingual region forms a semi-enclosed compartment in which solubilized allergens can be withheld without immediate swallowing for a limited period of time.[22]

The immunologic response to sublingually applied allergen extract is initiated by the uptake and processing of allergens and other antigens present in the extract by antigen-presenting cells, predominantly Langerhans cells, embedded in the oral epithelium.[32] Allergen uptake is likely a rate-limiting step in SLIT, as binding to the antigen-presenting cells has been shown to depend on both the allergen dose and the mucosal contact time.[32,33] Allergen dose and mucosal contact time are determining factors of allergen uptake and therefore also of the nature and magnitude of downstream immunologic tolerogenic events.[32] The importance of allergen dose and mucosal contact time for allergen uptake emphasizes that key attributes of any AIT SLIT-tablet must include the ability to deliver the full allergen content of the tablet into soluble form while at the same time maximizing the mucosal contact time of solubilized allergen.

The experiments presented in this article were designed to examine the primary properties of SLIT-tablet allergen release, disintegration time and dissolution rate in addition to tablet major allergen content, and recovery where applicable. Disintegration time shows the speed at which the tablet falls apart on contact with solvent in order to allow hydration of the allergen extract content, and dissolution rate is a direct measure of the amount of soluble allergen that becomes released from the dry tablets as a function of time.

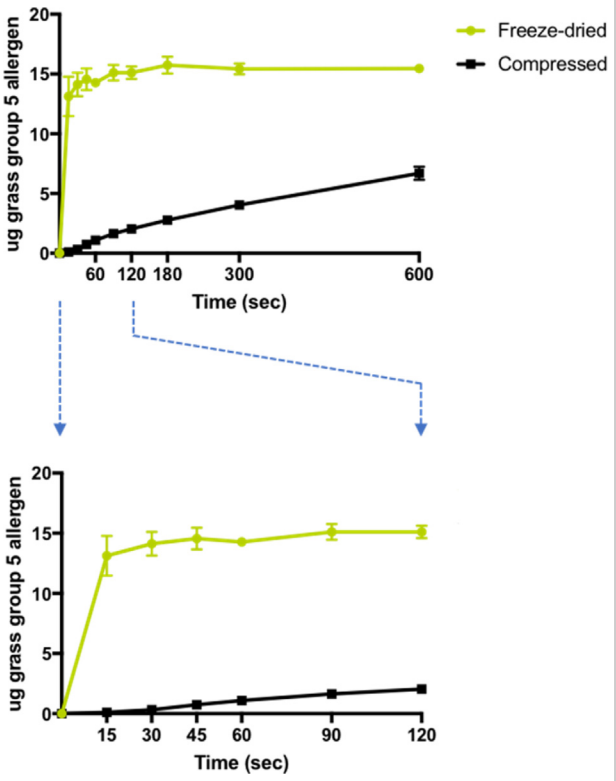


Figure 3. Allergen release kinetics (μg). The amounts of grass group 5 major allergen released into solution from the freeze-dried SQ grass SLIT-tablet and the compressed IR grass SLIT-tablet from 0 to 600 seconds (upper panel) and 0 to 120 seconds (lower panel) was measured by enzyme-linked immunosorbent assay using the 75,000 SQ-T in-house reference as a standard. The differences between the 2 tablets with regard to grass group 5 major allergen dissolution were significant ($P < 0.001$ at 15 seconds, $P < 0.0001$ at all other time points).

Preliminary experiments (data on file, Torii Pharmaceuticals study no. 125-069 2016) indicated that the release of soluble allergens from compressed SLIT-tablets was affected by the chemical composition of the solvent, with higher yields of soluble allergen being achieved in buffered saline solutions containing carrier protein than in purified water. This finding is in contrast to those with the freeze-dried SLIT-tablets, which performed equally well in water and buffered solutions with or without carrier protein when examined in parallel, in conditions identical to those with the compressed SLIT-tablets. Consequently, the assay buffer used in these experiments was designed to accommodate both SLIT-tablet formulations and furthermore to comprise a composition similar to human saliva with regard to pH value, ionic strength, and total protein content.[27]

When tablet disintegration time was measured, 2 prominent differences between the freeze-dried SQ grass SLIT-tablets and the compressed IR grass SLIT-tablets were noted. First, the freeze-dried SQ grass SLIT-tablets dissolved completely and rapidly on contact with solvent, without any noticeable disintegration process and, accordingly, a disintegration time of 1 second was recorded. In contrast, the compressed IR grass SLIT-tablets disintegrated gradually over a mean period of 31 seconds until the assay-specified criteria for disintegration were met. Second, while the freeze-dried SQ grass SLIT-tablets dissolved to an extent at which no tablet residue was evident, a substantial amount of undissolved particulate material was easily recovered by centrifugation after complete disintegration of the compressed tablets. The different disintegration properties do not *per se* provide any indications as to the effectiveness of the 2 SLIT-tablet types, but they demonstrate fundamental differences in the design of the tablet formulations. The extent and rate of allergen dissolution are, on the other hand, directly linked to the main purpose of SLIT-tablets, which is to release allergens from the dry state of the tablet into solution as efficiently as possible. Two parameters should be emphasized: (1) the extent of allergen solubilization compared to the total allergen content of the tablet, and (2) the rate of allergen dissolution. The ELISA assay used for grass group 5 quantification includes the SQ grass SLIT-tablet in-house reference as a standard, and although the SQ standardization procedure involves parameters in addition to Phl p 5 content, measurements of Phl p 5 released from the freeze-dried SQ grass SLIT-tablet using this assay are directly comparable to the nominal strength of the tablet and can be expressed as SQ-T Phl p 5 equivalents. The ELISA value obtained after dissolving the freeze-dried SQ SLIT-tablet in 1 mL of assay

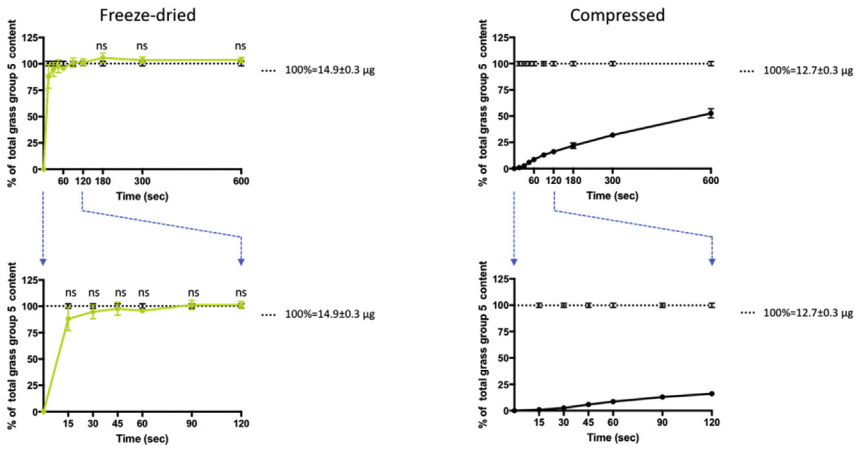


Figure 4. Allergen release kinetics (relative). The amounts of grass group 5 major allergen released into solution from the freeze-dried SQ grass SLIT-tablet (left panels) and the compressed IR grass SLIT-tablet (right panels) were measured by enzyme-linked immunosorbent assay, using the total grass group 5 major allergen values from the Table as references. No statistically significant differences between the amounts of released allergen and the reference values were seen at any time with the freeze-dried tablet. With the compressed tablet, the amounts of released allergen were statistically significantly different from the reference values at all time points ($P < 0.001$) (data not shown). ns = nonsignificant.

buffer was 74,675 (1623) SQ-T Phl p 5 equivalents (mean [SD]; n = 4) (data not shown). This finding demonstrates that in these conditions, full recovery (99.6% [2.2%]) of the total Phl p 5 content of the 75,000 SQ-T freeze-dried SQ grass SLIT-tablet was achieved. With a Phl p 5 content of 15 µg/75,000 SQ-T,[34] 99.6% corresponds to a recovery of grass pollen major allergen of 14.9 (0.3) µg.

Optimization of dissolution was more challenging with the compressed IR grass SLIT-tablet than with the freeze-dried SQ grass SLIT-tablet, and a different and more intensive procedure was used to reach the, in our hands, maximal amount of retrievable soluble grass group 5 allergens. Since the drug substance used for the compressed IR grass SLIT-tablet was not available, it was not possible to make an accurate calculation of the relative recovery of solubilized grass group 5 allergen following tablet dissolution compared to the complete tablet allergen content, but the amount of solubilized grass group 5 major allergen obtained from the compressed IR grass SLIT-tablet can be measured by ELISA and was determined to be 12.7 (0.3) µg (Table). This value is less than the published 25 µg grass group 5 allergen/300 IR,[34] but since quantification of allergen concentration by biological assays is known to be sensitive to the antibodies and standards used in the respective assays, the 2 values may not be comparable. However, since the same assay is used here, the 14.9 (1.6) and 12.7 (0.3) µg of solubilized grass group 5 major allergen obtained with the freeze-dried SQ grass SLIT-tablet and the compressed IR grass SLIT-tablet, respectively, are comparable. Although the measured values of grass group 5 major allergen content of the 2 tablets were statistically significantly different, the numbers indicate similar yields of solubilized grass group 5 major allergens from the 2 tablets, with the caveat that a more intensive procedure was needed to extract the grass group 5 content from the compressed IR grass SLIT-tablet. A factor that could have affected the quantification of grass group 5 major allergen by ELISA is that the freeze-dried SQ grass SLIT-tablet contains extract from a single grass species, *Phleum pratense*,[35] whereas the compressed IR grass SLIT-tablet contains a mixture of 5 closely related but nonidentical species (*Phleum pratense*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis*, and *Anthoxanthum odoratum*).[36] The presence of additional species in the compressed IR grass SLIT-

tablet tablet could cause a potential bias in terms of allergen quantification due to species-specific allergen variants. However, the antibodies used for the ELISA have been shown to react equally well with *P pratense* as with *D glomerata*, *L perenne*, *P pratensis*,[29] and *A odoratum*.

The 2 tablet formulations differed markedly with regard to grass pollen major allergen release profiles, both with regard to release rate and yield of soluble allergen. While the initial release rate of the freeze-dried SQ grass pollen SLIT-tablet was very high, the allergen release rate of the compressed IR grass SLIT-tablet was almost linear for the duration of the experiment, and no leveling off of the release curve was observed, indicating incomplete allergen release even after 10 minutes in solution. When illustrated on a relative scale in which the amounts of grass group 5 major allergen in the Table were regarded as maximal allergen release, that is, 100% = 14.9 and 12.7 μg with the freeze-dried SQ and compressed IR grass SLIT-tablets, respectively, a similar pattern was seen. Complete allergen release was reached within 15 seconds with the freeze-dried SQ grass SLIT-tablet, but only 15.8% of the grass group 5 major allergen content shown in the Table was released from the compressed IR grass SLIT-tablet after 120 seconds in solution, increasing to 51.8% after 600 seconds, when the experiment was stopped (Figure 4). It is of course possible that 12.7 (0.3) μg of grass group 5 major allergen shown in the Table represents an underestimate of the actual grass group 5 allergen content of the compressed IR grass SLIT-tablet, but even so, the amounts of released grass group 5 allergen were below the measured total grass group 5 allergen content shown in the Table, and therefore, at all time points, incomplete recovery of soluble allergen was achieved with the compressed IR grass SLIT-tablet in the same experimental conditions in which complete allergen release was rapidly achieved with the freeze-dried SQ grass SLIT-tablet. When the allergen release kinetics and yields of the 2 tablets are illustrated on a relative scale, with the maximal yields of soluble allergen from individual tablets as references, the data are independent of any differences between the tablets with regard to the respective grass extract species and allergen compositions.

The allergen release profiles shown here may not be directly translatable to the *in-vivo* situation in which a SLIT-tablet is placed under the tongue and left to dissolve and release soluble allergens into a small volume of saliva with little agitation, but they are still illustrative of the differences in the performances of the compressed and freeze-dried SLIT-tablet formulations. The freeze-dried SQ grass SLIT-tablets disintegrated and released soluble allergen quantitatively in a robust and reproducible manner independent of assay conditions. In contrast, allergen release rates and yields of soluble allergen of the compressed IR grass SLIT-tablets were sensitive to assay conditions such as solvent composition, solvent volume, and intensity of agitation. The dependency on assay conditions for solubilization of allergen from the compressed SLIT-tablet formulation is particularly clear from the fact that the yield of soluble allergen achieved with the compressed IR grass SLIT-tablet in one set of conditions was not met, at any time point, in another, less intensive, set of conditions.

Despite the differences in tablet formulations, both the freeze-dried 75,000 SQ-T tablets and the compressed 300 IR grass pollen SLIT-tablets have been reported to be clinically efficacious in large-scale clinical trials.[3,4] However, an important difference between the 2 tablets that is not readily discernable from their respective nominal strengths is the allergen content. The SQ-T and IR units relate to different assays and allergen standards and are therefore not directly comparable, but when compared based on major allergen content (25 μg of major allergen in the 300 IR compressed tablet, 15 μg in the 75,000 SQ-T freeze-dried tablet[34]), or standardized against a common allergen extract standard as in the United States and Japan, the differences become more obvious. In the United States, the nominal strengths of all AIT products are assigned by establishing the potency of the preformulation drug substances in bioequivalent allergy units (BAU) using common assays and standards, and here the freeze-dried 75,000 SQ-T grass SLIT-tablet and the compressed 300 IR grass SLIT-tablet were assigned nominal strengths of 2800 and 9000 BAU, respectively.[37,38] However, while the freeze-dried 75,000 SQ-T/2800 BAU grass SLIT-tablet has been reported to be clinically efficacious,[4,39] the compressed 100 IR/3000 BAU grass SLIT-tablet has not,[40] despite the higher nominal potency in BAU. In Japan, AIT product standardization is measured in JAUs using common assays and reagents, and while a compressed 100 IR/19,000 JAU HDM SLIT-tablet was not found to be

clinically efficacious,[41] 2 freeze-dried HDM SLIT-tablets with nominal strengths of 6 SQ-HDM/10,000 JAU and 12 SQ-HDM/20,000 JAU both demonstrated robust clinical effect in large-scale Phase III trials,[7,8] despite a lower or similar nominal strength in JAU. Why the freeze-dried SLIT-tablets with nominal strengths of 75,000 SQ-T/2800 BAU, 6 SQ-HDM/10,000 JAU, and 12 SQ-HDM/20,000 JAU have all been found to be clinically efficacious while their compressed counterparts of equal or higher BAU or JAU potencies have not, is a conundrum. However, the lack of clinical performance of the compressed 100 IR SLIT-tablets despite higher allergen content may be explained by less-efficient allergen delivery compared to the freeze-dried SLIT-tablets, which is in line with the data presented here and in a previous publication.[42]

The extent to which different allergen release profiles affect the downstream immunologic and clinical effects of the freeze-dried SQ grass-SLIT-tablet and the compressed IR grass SLIT-tablet in a standard clinical treatment regimen cannot be determined without further experiments designed for this purpose. Nonetheless, SLIT-tablet efficiency, with regard to allergen release kinetics and yield of soluble allergen, is likely essential for optimal SLIT-tablet performance, given the limited mucosal contact time recommended by the manufacturers and the fact that continuous dilution of the allergen by saliva occurs throughout the sublingual holding period by a process described as "saliva washout."[30] We speculate that in a case in which 2 SLIT-tablets containing the same amounts of allergen extract of identical composition and potency are held under the tongue for the same amount of time, fast and complete allergen release constitute the most efficient form of allergen delivery. Fast and complete allergen release will lead to the highest concentration of allergen on the mucosal surface for the longest time, compared to slower, continuous, and incomplete release. In SLIT, the true efficiency of allergen delivery likely should be measured as the allergen concentration and mucosal contact time in combination.[42] It is therefore conceivable that prolonged mucosal contact time and/or increased allergen dose could compensate for a less efficient SLIT-tablet allergen release profile, although the gain of prolonged mucosal contact time must be expected to be offset by allergen dilution due to continuous saliva production. Also, a treatment regimen that depends on longer sublingual holding times on a daily basis would be less convenient for patients, and expectedly less robust when considering a full 3-year treatment regimen. If a higher allergen dose in a slow-releasing SLIT-tablet is required to achieve a clinical effect similar to that of SLIT-tablets with more efficient allergen release, the total daily allergen load will increase, with an elevated risk for allergen-mediated side effects. The discrepancy with regard to clinically efficacious doses of the 2 SLIT-tablet formulations furthermore has the implication that nominal strengths of grass SLIT-tablets cannot be compared unless the tablets are based on the same formulation.

The data presented here and previously[42] clearly demonstrate that in the experimental conditions used, the release of allergen from the compressed IR SLIT-tablet is slower and incomplete compared to that from the freeze-dried SQ grass SLIT-tablet, and with the influence of factors such as solvent volume, time in solution, intensity of agitation, as well as the composition of the solvent. Based on the tablet disintegration and allergen release properties demonstrated here with the freeze-dried SQ grass SLIT-tablet and elsewhere with the SQ HDM SLIT-tablet,[42] the fast-dissolving freeze-dried SQ grass SLIT-tablet formulation may be an efficient vehicle for allergen delivery that can be expected to provide complete allergen release and deliver the full allergen potency of the tablet in soluble form consistent with maximal biological uptake.

## CONCLUSIONS

In this *in-vitro* study, the freeze-dried SQ grass SLIT-tablet provided efficient allergen delivery, including complete and rapid release of allergen into solution from the dry state of the tablet. Compared to the compressed IR grass SLIT-tablet, the freeze-dried SQ grass SLIT-tablet disintegrated faster, displayed faster allergen release kinetics, and achieved complete allergen release within 15 seconds in solution. Both grass SLIT-tablets are clinically efficacious, but the clinical effect of the freeze-dried SQ SLIT-tablet is achieved with a lower allergen content, which is possibly attributable to the high efficiency of allergen delivery provided by the freeze-dried formulation.

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## CONFLICTS OF INTEREST

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K. Lund, M.B. Skydtsgaard and S. Lawton own stock in ALK-Abelló A/S. Dr. Lawton is an employee of ALK-Abelló A/S. H. Kito, H. Nakazawa, and K. Ohashi-Doi are employees of Torii Pharmaceutical Co Ltd. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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