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# Evaluation of stability of allergen extracts for sublingual immunotherapy during transport under unfavourable temperature conditions with an innovative thermal insulating packaging

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## KEY WORDS

*Allergen extracts, quality, stability, Staloral, sublingual immunotherapy, temperature excursions, transport*

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## SUMMARY

*Many pharmaceutical and biotechnological products are temperature-sensitive and should normally be kept at a controlled temperature, particularly during transport, in order to prevent the loss of their stability and activity. Therefore, stability studies should be performed for temperature-sensitive products, considering product characteristics, typical environmental conditions, and anticipating environmental extremes that may occur during product transport in a specific country. Staloral products for sublingual immunotherapy are temperature sensitive and are labelled for maintenance under refrigerated conditions (2–8°C). Given the peculiar climatic context of Italy and the great temperature fluctuations that may occur during transport, this study was aimed at evaluating the impact of a new engineered thermal insulating packaging for Staloral. In particular, the purpose was to assess whether the new packaging could create a container condition able to preserve the stability and immunological activity of the product during the transport phase throughout Italy. The results showed that the range of temperatures that can affect the product, in the area surrounding the product packaging, may reach a peak of 63°C during transport under the most unfavourable climatic conditions, i.e. in a non-refrigerated van during the summer season, from the site of production in France to the patient's house in Catania, the city with the highest temperatures in Italy. However, the highest temperature reached inside the vaccine did not exceed 45°C over a period of about 2 h. The ELISA inhibition test on samples subjected to the extreme temperature conditions previously defined (45°C) showed an immunological activity higher than 75% of that initially measured and was comparable to those obtained with samples stored at controlled temperature (5°C). This means that, even in the worst case scenario, the structure of the allergen extracts is not influenced and the vaccine potency is preserved.*

## Introduction

An ever-increasing number of pharmaceutical and biological products prescribed worldwide – such as insulin, vaccines, biologicals, chemotherapeutic agents, blood products, antibiotics – are temperature sensitive. For this reason, they require special production, packaging, transport, and storage conditions to avoid their exposure to inappropriate environmental factors in order to guarantee the maintenance of their stability. This issue is of paramount importance, since the preservation of product stability relates to the preservation of its quality – and hence its therapeutic properties, i.e. safety and efficacy – when finally used by patients (1).

It is well known that the properties of a pharmaceutical product depend on the features of all the molecules it contains and that these molecules can exert their activity when they have a certain chemico-physical structure. However, specific environmental parameters can influence the structural integrity of the molecules and can therefore impair their functional activity. Therefore, it is particularly important to preserve a pharmaceutical product in order to preserve its beneficial effects during treatment (2-3).

For instance, temperature is a critical parameter for given products, since inappropriate temperature fluctuations can cause conformational instability of proteins and produce a marked decrease in the quality of such products (4,5). So-called stability studies are therefore essential during the pharmaceutical development process, with the purpose of completely understanding the product characteristics and the impact that specific environmental factors (such as temperature) could possibly have on product stability (6-11). The ultimate purpose of such studies is to determine the optimal conditions that should be considered for the handling of the pharmaceutical product itself; moreover, with this type of studies, it is possible to predict the expiry date and shelf life of the product, if stored correctly, and thus to suggest appropriate labelling instructions (4,12,13).

One of the major concerns of the pharmaceutical industry and health authorities is that products should be delivered to patients without loss of therapeutic properties, preserving their activity up to a level of 85% of that claimed on the label (1,12,13). Indeed, it is noteworthy that regulatory guidelines set up and recently updated by World Health Organization and European Commission, generally known as “Good Distribution Practice” (GDP), focussed on this issue and highlighted the value of preservation of product stability in the entire supply chain

(14,15). Therefore, in order to be compliant with the GDP guidelines for the transport of temperature-sensitive products, a pharmaceutical company should perform a distribution stability study, to complement routine general stability experiments performed during the developmental phase, as already described. A good protocol for this purpose would be to expose the pharmaceutical product to a setting that mimics, as far as possible, the foreseeable outdoor conditions, with temperatures that are higher and/or lower than those recommended for storage, and thus to evaluate the functional parameters reflecting possible changes in the product activity. The combined data could thus provide information necessary for supporting certain nonconformities to labelling – with a certain margin of deviation allowed for a limited duration and within limited range – or to improve protective packaging and container conditions, developing product-shipping criteria, and validating the overall robustness of the distribution program (1,4,16).

The aim of the present study was to investigate the impact of an innovative and engineered thermal insulating packaging, developed for the transport of Staloral products, which are allergen extracts for sublingual immunotherapy (SLIT) in patients with respiratory allergies (17-21). In particular, we aimed to establish whether the new packaging could successfully create container conditions capable of preserving the stability and immunological activity of the product itself during the transport phase throughout Italy, across a range of unfavourable temperature conditions different from those mentioned on the label for storage (a range of 2-8°C). This distribution stability study with the new packaging was planned in order to confirm the maintenance of product quality in the supply chain in Italy and hence the compliance to GDP guidelines.

Italy is characterized by a peculiar climate; it is mainly Mediterranean, but nonetheless presents marked variability of temperatures from north to south, and shows temperatures that are very different from one season to the other. Moreover, Staloral products belong to a category of products generally referred to as “named patient product” (NPP), since they are prescribed by the physician specifically for a given patient and transported one by one from the production site directly to the patient’s house, making the logistics of transport even more complex. In addition, the immunologically relevant allergens in the Staloral products are subjected to loss of potency over time due to improper temperatures and are hence to be always maintained in cold-chain systems, refrigerated between 2-8°C, to ensure their stability.

## Material and methods

### *Biopharmaceutical products*

The thermal stability studies described in the present paper were performed using Staloral products. The three types of extracts that are most commonly used in Italy were analysed: a mix of 5 grass extracts at the concentrations of 10 IR and 300 Index of Reactivity (IR); a mixture of 2 mite extracts (*Dermatophagoides pteronyssinus/farinae*) at 10 IR and 300 IR; and *Parietaria officinalis* extract at 100 IR and 300 IR.

### *Detection of climatic data*

A study was carried out in order to identify the Italian areas with the highest summer temperatures and the worst general climatic conditions. The Osservatorio Meteorologico Milano Duomo (OMMD) was asked to collect and analyse the daily data related to all the provinces in every region of Italy. For each city, for each of five summer seasons from 2002 to 2006 and for each of five winter seasons from 2001/2002 to 2005/2006, the following data were taken into account: maximum annual temperature (with the date); minimum annual temperature (with the date); maximum value of the current mean of eight days of daily maximum temperature (with start date and end date); for the eight days determined, the average trend of daily temperature on an hourly basis (24 values); and minimum value of the current mean of eight days of daily minimum temperature (with start date and end date). Catania was found to be the most unfavourable Italian city from the point of view of temperature, due to the maximum temperatures reached in summer.

### *Mathematical assessment of temperatures inside the van*

Product packaging was defined as the thermal insulating packaging surrounding the vial of Staloral; an analytical calculation was set up to define the maximum temperature that can be reached in the area surrounding the product packaging when it is inside a non-refrigerated van, such as a Fiat Iveco. Analysis was based on climatic data of the most unfavourable region in the summer season (Catania) and on direct solar radiation for the period during which delivery can occur (from 8.30 to 13.00), as previously found by OMMD. Firstly, the calculation was performed considering that the product packaging inside the van is subjected to solar radiation transmitted from

the walls of the van itself. As a second step, a situation more appropriate to reality was modelled, given that the product packaging is transported together with other packages and therefore at least three of the faces of the parallelepiped forming the packaging (the two vertical faces and the lower face that rests on the floor of the van) are shielded.

### *Mathematical assessment of temperature range during transport*

A time-temperature diagram was prepared, based on the collected data. It reproduces the temperature range that may affect the product packaging from time of packing (Paris, France, where the production site is) until time of delivery (Catania, Italy, where the patient's house is in theory) during summer. The outdoor temperature ("To", Temperature outdoor) was defined as those analysed by OMMD for the city of Milan (northern Italy), Florence (central Italy), and Catania (southern Italy). The temperature in the area surrounding the product packaging was called the surrounding temperature ("Ts", Temperature surrounding). When in France, it was assumed prudently that the maximum temperatures that can potentially arise outside the product packaging did not exceed 25°C.

### *Laboratory test no. 1: establishing the temperature in the product*

Laboratory test no. 1 was set up in order to reproduce, as closely as possible, the expected time-temperature scenario and to verify experimentally the temperature that is reached inside the vaccine during the shipment to the patient's house during the warm season. The test was performed at the *Laboratorio Prove Materiali* (LPM) of the *Politecnico di Milano* (PM) in a climatic chamber where a thermal transient as near as possible to the diagram of the temperature "Ts" assessed in the previous section was replicated. The simulation test was carried out by placing a vial of Staloral in its thermal insulating packaging inside the climatic chamber, with three of its faces shielded by walls made up of panels of polystyrene in order to simulate the presence of two additional packages during transport in the van and to take account of the fact that the surface resting on the floor is not subjected to solar radiation. Three separate thermocouples were placed inside the climatic chamber: one outside the product packaging to obtain a real-time reading of the temperature in the surrounding area ("Top", temperature outside the packag-

ing); one inside the thermal insulating packaging, but outside the product vial (“Tip”, temperature inside the packaging); and one inside the product vial to evaluate the temperature reached inside the vaccine itself (“Tv”, temperature inside the vaccine).

### *Experimental assessment of temperatures during transport*

The product packaging was shipped in order to evaluate all the assumptions made above in actual practice. The product packaging was transported from Milan to Catania inside a van during the summer (July 3, 2007). During the shipment, the temperatures that were reached outside and inside the product packaging were all noted down.

### *Laboratory test no. 2: assessing the biological stability of the vaccine*

Laboratory test no. 2 was arranged to reproduce the same condition as laboratory test no. 1 on 402 vials of Staloral in their thermal insulating packages, in order to allow them to be subjected to the following biological stability studies. The simulation test was performed at the LPM of the PM, in the same climatic chamber used in laboratory test 1. The vaccines were given to the PM ensuring that samples had been maintained at 2–8°C, from the moment of leaving the production site in France to the moment of delivery. Once in the laboratory, samples were first positioned on metal grilles before being placed in the climatic chamber; this operation was carried out at 25°C, in order to have vaccine samples at a temperature close to the outdoor temperature. The thermal transient applied was the “Tv” of test no. 1. The product packages were then placed in the refrigerator at 2–8°C and sent, at controlled temperature, to Stallergenes, France, for biological stability studies.

### *Assessment of biological stability of the vaccine*

The power of allergenic extracts exposed to conditions reproduced in laboratory test no. 2 was then measured in the Stallergenes laboratory, under the supervision of the Quality Control Department. The purpose of this part of the study was to evaluate whether the biological stability of the vaccine was impaired by the temperature fluctuations experienced in the previous section (reproducing the temperature range during the transport of the product packaging in the van). The allergenic activity was evaluated by using an ELISA inhibition test *in vitro*. A value of inhibition > 75% of the initial allergenic activity value was

taken as an adequate indication of good preservation of the vaccine. Samples of the vaccine that had been stored in the refrigerator at 5°C for the same period of time were used as a control for this study.

### *Laboratory test no. 3: assessing the engineered packaging*

Based on the good outcome of the biological stability study, the prototype of packaging used previously was engineered. This led to the development of an innovative thermal packaging in Neopor® (produced by Basf, Germany) with greater thickness, but without the external reflective film, and with optimized dimensions for the containment of one or two boxes of the product. With the new packaging, laboratory test no. 3 was performed at the LPM of the PM, using the same climatic chamber. The temperatures reached inside the vaccine when the product packaging was subjected to an outside temperature range similar to that used in test no. 1 were then measured. The aim was to compare the thermal insulating capacity of the new packaging with that of the prototype.

## **Results**

### *Assessment of temperature range during transport*

#### *Mathematical modelling*

Table 1 and the corresponding time–temperature diagram (Figure 1), mathematically modelled, show that 63°C is the highest temperature that can influence the temperature “Ts” during transport under the assumed unfavourable conditions.

#### *Simulation testing in the climatic chamber*

Laboratory test no. 1 in the climatic chamber demonstrated experimentally that, even if peaks of 63°C were applied to product packaging (temperature “Ts”, as determined in the previous mathematical modelling), the maximum temperature “Tv” did not exceed 45°C over a period of 2 h (Figure 2). This suggested good thermal capacity of the packaging.

#### *Shipping in real practice*

With shipping in real practice (Figure 3), we found that, even considering a “Top” temperature in Catania in the

**Table 1** - Temperature range affecting product packaging from production until delivery to patient

	Time (h)	Ts (°C)	To (°C)
<b>First Day (France)</b>			
Boxes withdrawal from Gennevilliers	15:30	25.0	25.0
N.B.: allergen extract is packaged at 2-8°C			
Arrival at Paris among international carrier (FedEx)	00:00	24.9	25.0
Arrival at Orly	03:00	23.2	25.0
Flight from Orly to Milan	04:00	14.0	25.0
<b>Second Day (North and Center Italy)</b>			
Arrival at Malpensa airport in Milan	05:30	14.0	25.0
Arrival on carrier's storage place (Sesto Ulteriano)	07:30	25.0	27.8
Boxes delivering at Stallergenes's storage place (Caleppio di Settala)	08:30	25.0	29.3
Packaging of vaccine in polystyrene boxes	09:30	25.0	30.6
Boxes withdrawal by a carrier	14:30	35.0	36.3
Transport in van to the sorting center	15:00	42.0	36.9
Boxes unloading among carrier branch	17:30	47.0	37.2
Boxes processing	18:00	35.0	36.3
Bar code reading	18:30	35.0	36.3
Boxes sorting according to destination	19:30	35.0	35.0
Loading on tracks	20:00	35.0	33.5
<b>Third Day (South Italy)</b>			
Arrival to terminal	06:00	24.2	23.3
Picking up by local carrier	10:00	38.0	32.6
Boxes return in case of failed delivery	13:00	63.0	36.4
Storage among terminal	13:30	33.0	36.4
<b>Fourth Day (South Italy)</b>			
Continuous storage among terminal	08:30	27.0	27.3
Picking up by local carrier	10:00	38.0	32.6
Delivery to patient	13:00	63.0	36.4

Ts: surrounding temperature; To: outdoor temperature

order of 33°C, the highest "Tip" temperature reached during transport in the van was in the order of 38°C. Therefore, the thermal insulating packaging showed good thermal inertia, as evaluated over a period of 4 h.

#### ***Assessment of biological stability as a function of temperature range***

##### *Biological stability studies*

The results obtained with the samples subjected to biological stability tests were as follows. For the mix of five grass extracts, the values of inhibition derived from the ELISA test were 109% for the 10 IR concentration and 83% for the 300 IR concentration, compared to 101% and 85% of the control extracts. For the mix of two mite ex-

tracts, inhibition values were 95% for 10 IR and 111% for 300 IR, as compared to 97% and 114% for the controls. For *Parietaria*, inhibition values were 84% for 100 IR and 96% for 300 IR, as compared to 89% and 93% for the controls (Figure 4). The values obtained for the tested samples demonstrated an immunological activity higher than 75% of the activity initially measured; moreover, these values were not statistically significantly different from those of the control samples.

##### *Validation of the thermal insulating packaging during transport*

With laboratory test no. 3, the temperatures reached inside the vaccine in the newly engineered packing material were

Figure 1 - Temperature range affecting product packaging from production until delivery to patient

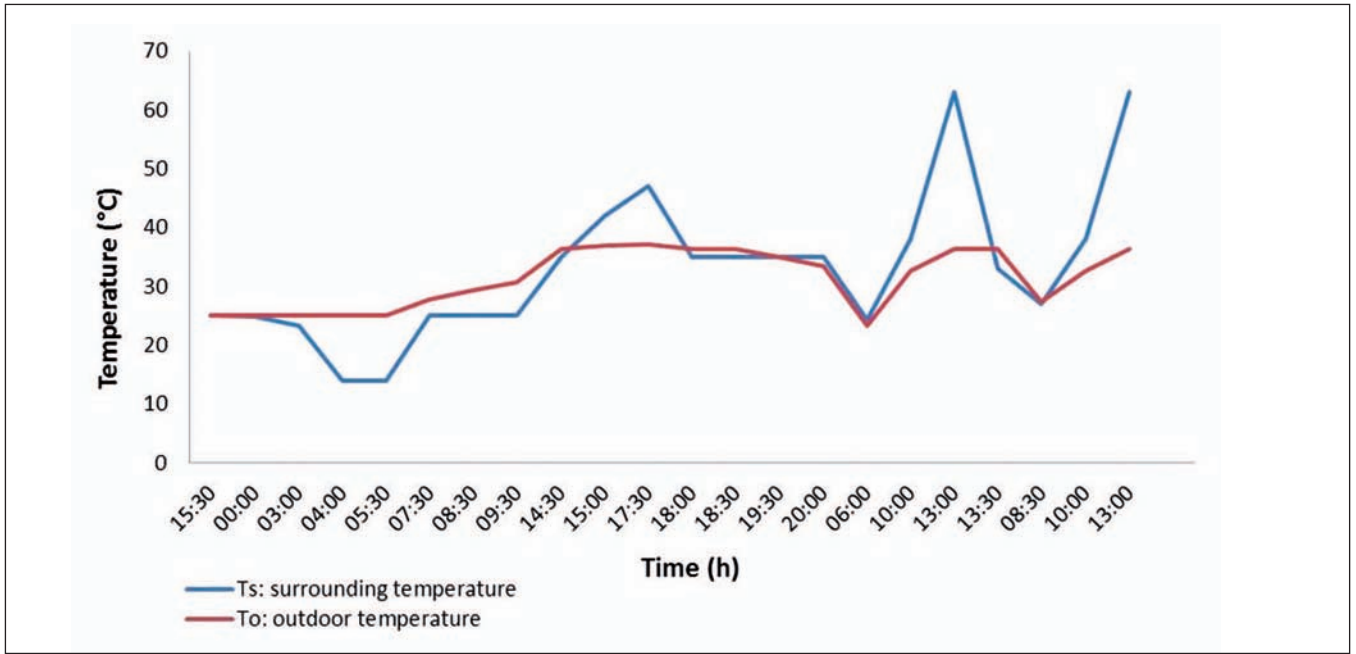
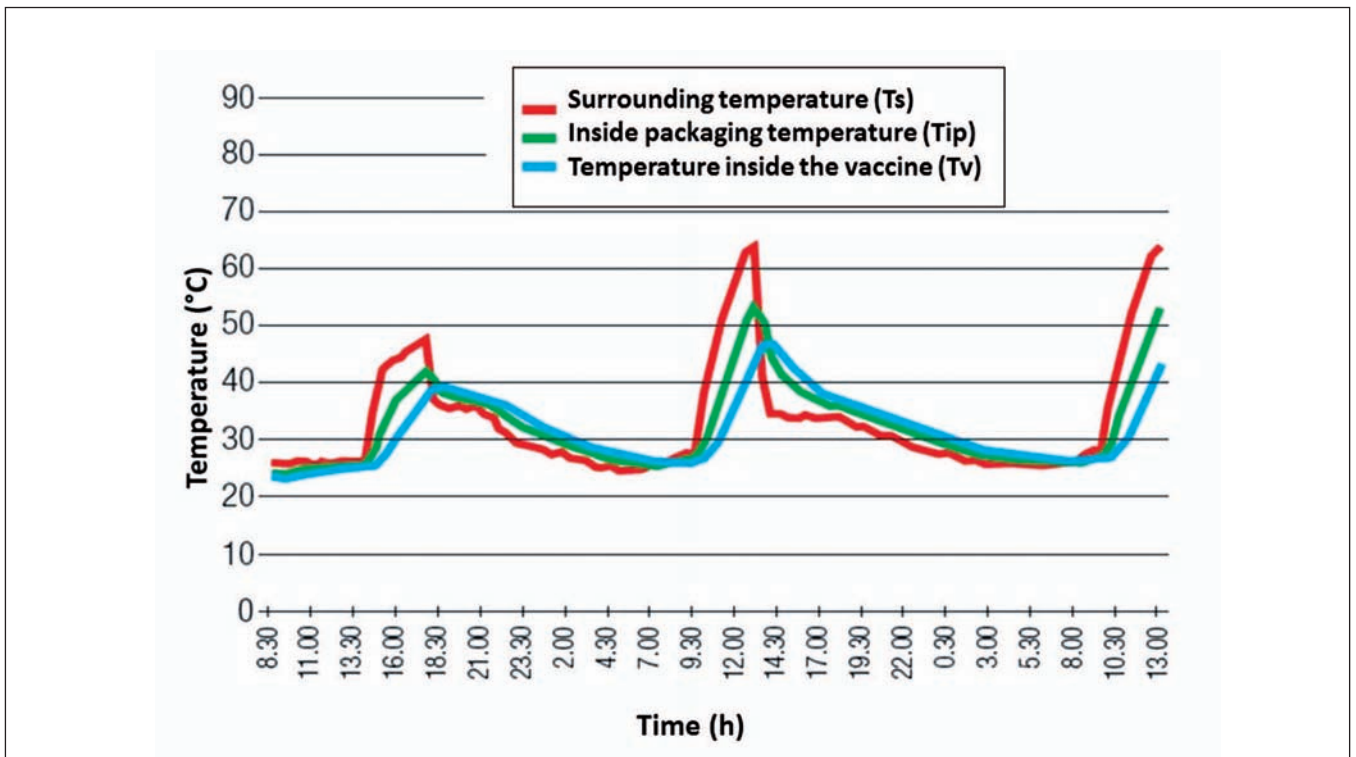
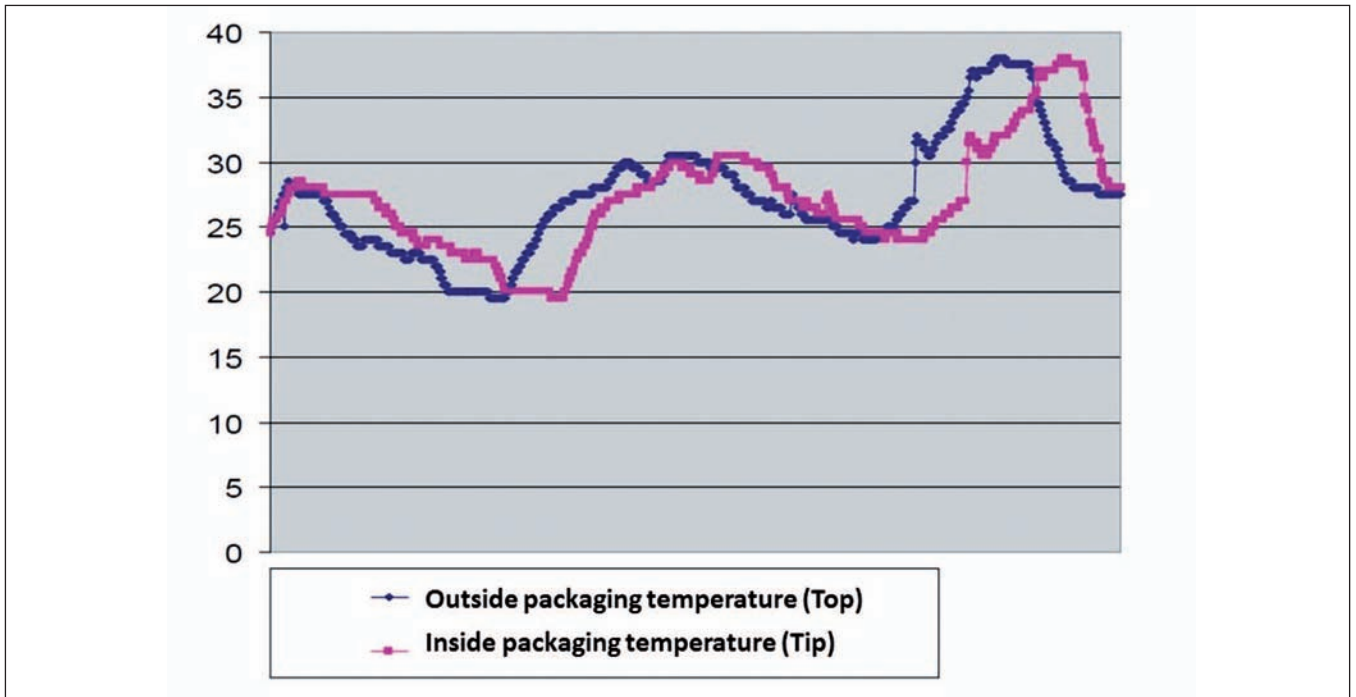


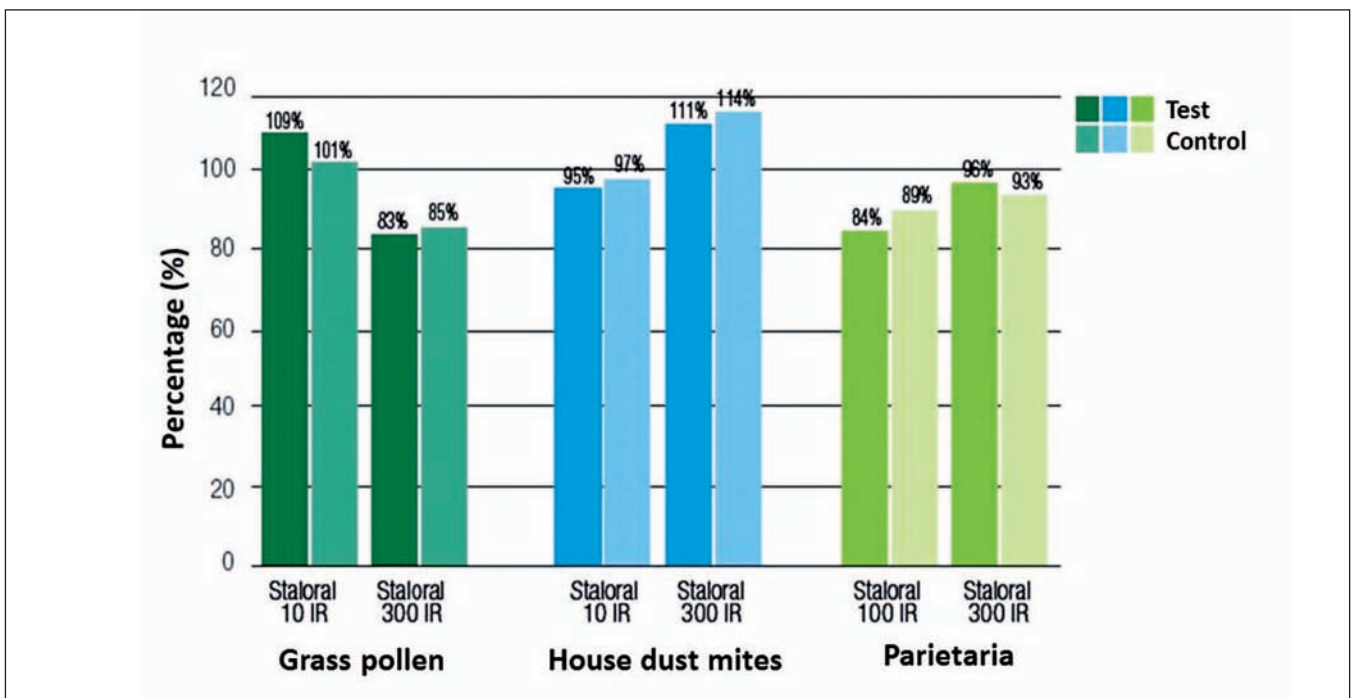
Figure 2 - Assessment of the temperature range which can affect the product packaging, performed in the climatic chamber of the PM during the laboratory test no. 1



**Figure 3** - Assessment of temperatures inside and outside the Staloral packaging during a real shipment from Milan to Catania in the summer season



**Figure 4** - Assessment of immunological activity of product samples subjected to the thermal transient and of product samples maintained at a controlled temperature and used as a control



compared with those reached in test no. 1 with the vaccine in the prototype packaging material (Figure 5), and there was clear overlap of results between the two tests. Within the framework of a time-shift, due to different thermal inertia between the prototype and production packaging, the temperatures reached in the vaccine (“Tv1” temperature inside the vaccine in test no. 1, and “Tv3” temperature inside the vaccine in test no. 3) were substantially similar.

## Discussion

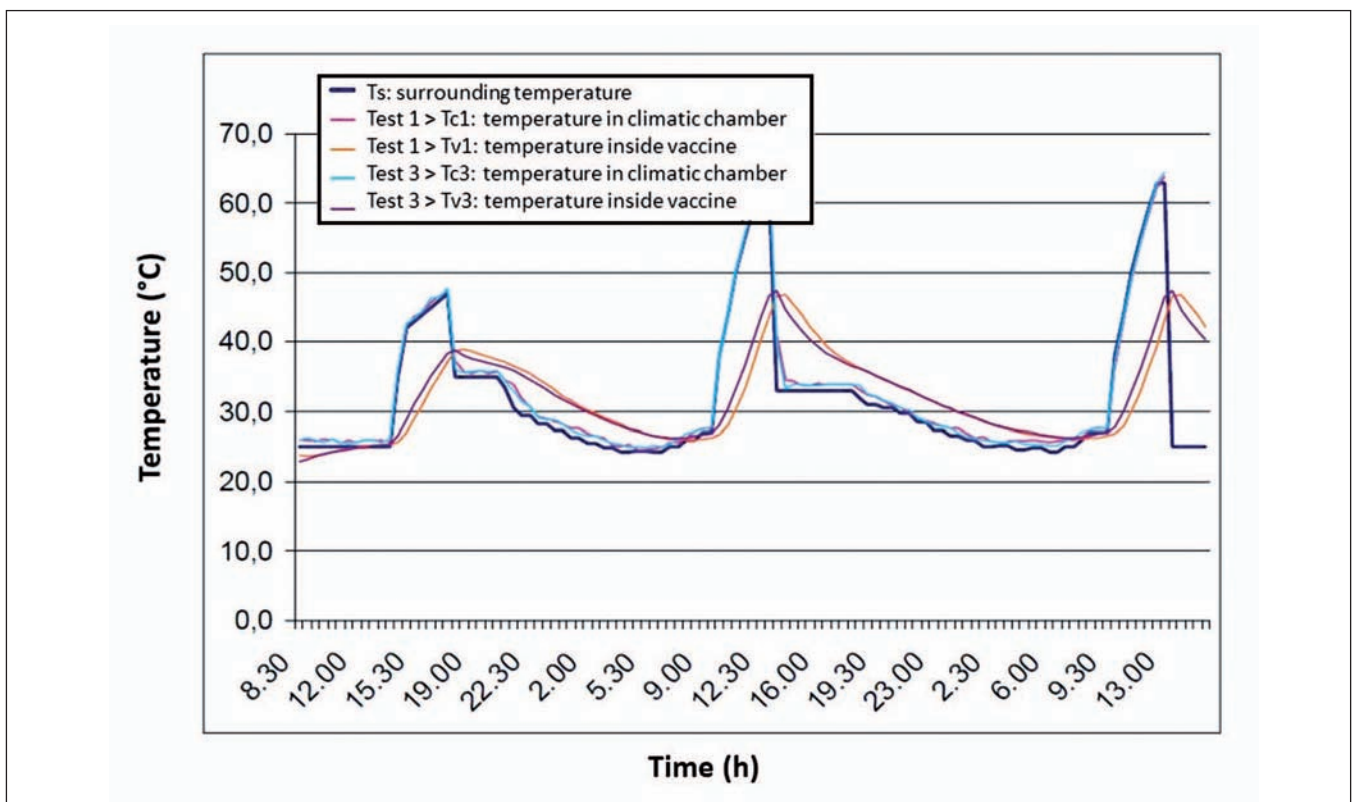
Staloral products are made up of allergen extracts prescribed to be stored in a refrigerated environment, between 2–8°C. High temperatures, indeed, are regarded as a factor impairing the stability of the immunologically relevant allergens, capable of altering the quality and hence the potency of the vaccine itself.

Italy is characterized by a peculiar climate with marked temperature variations from north to south and from cold

season to warm season, making it challenging to retain full control over this parameter during transport. Moreover, Staloral products are NPPs and are transported individually from the production site to the patient’s house, making the logistics of transport even more complex. Therefore, the present study was performed in Italy in order to assess the preservation of stability of Staloral products if transported inside an innovative thermal insulating packaging, which was developed to improve the distribution operation conditions and to better guarantee the quality of the delivered vaccine, in line with GDP principles. In particular, the project was used to evaluate whether, in the packaging described, the allergen extracts comprising Staloral products—differently from other types of temperature-sensitive products—could safely be transported outside of the cold-chain and eventually support transport even in the presence of excessive climatic heat, for a limited duration and within a limited range.

Mathematical modelling initially showed that the range of temperatures (“Ts”) affecting the product can reach

**Figure 5** - Assessment of the temperature range that can affect product packaging, conducted in the climatic chamber at the PM during the laboratory test no. 3





peaks of 63°C during transport under the most unfavourable climatic conditions, i.e. in a non-refrigerated van during summer, from the production site in France to the patient's house in Catania, the city with the highest temperatures in Italy.

In the climatic chamber, it was found that the highest "Tv" temperature did not exceed 45°C over a period of about 2 h, which corresponded with the 63°C peak of "Ts", if the product was kept inside the packaging with defined heat-insulating characteristics.

Finally, summer transport, in real practice, from Milan to Catania provided further evidence for the efficacy of the packaging. It showed a good thermal inertia, valued at approximately 4 h, since the highest "Tip" temperatures reached during transport in the van were around 38°C, whereas the "Top" temperatures detected in Catania were about 33°C. Interestingly, during transport in the van, the highest temperatures conservatively assumed in theoretical terms were not achieved. This is because the theoretical modelling deliberately did not take into account the following factors that are known to reduce "Ts": the contribution of the thermal inertia of the other items during transport; the non-overlapping highest summer temperatures in the localities passed through during transport and storage; and the shadow that protects against sunlight during some phases of the distribution process.

The ELISA inhibition test on samples subjected to the extreme temperature conditions previously defined (45°C) showed an immunological activity in excess of 75% of that initially measured and overlapping results when compared to those obtained on samples stored at controlled temperature (5°C). This means that, even in the worst case scenario—with higher temperatures than those realistically expected—the structure of the allergen extract is not influenced and the vaccine potency is preserved.

Since the biological stability analysis yielded positive results, the prototype of the thermal insulating packaging used in the earlier parts of the study was engineered to improve the conditions for product transport. Laboratory test no. 3 was then arranged in the climatic chamber using this new packaging, reproducing a temperature range comparable to that used in test no. 1 with the packaging prototypes. This test showed a similar behaviour of the new packaging. Moreover, as observed by the overlap of the diagrams of "Ts" temperatures inside the van and those in the climatic chamber in test no. 3 ("Top 3"), the packaging was subjected to a range of temperatures with higher peaks than those realistically expected; therefore, the results should be considered conservative.

In conclusion, these data demonstrated that the engineered packaging has a good thermal capacity for maintaining proper thermal insulation; moreover, these experiments established that Staloral allergen extracts for SLIT, if transported in this new packaging, preserve optimal characteristics even under temperature fluctuations, thus ensuring a safe and effective treatment for allergic patients. Therefore, this study proved that the distribution process arranged for these products is appropriate and compliant with GDP principles.

### Acknowledgements

The authors thank Perucci Engineering (Milan) for technical support in mathematical and statistical analysis and Politecnico di Milano (Dipartimento di Ingegneria strutturale, Laboratorio prove materiali) for technical assistance with the experiments in the climatic chamber.

### Funding

Stallergenes Italy funded the study.

### Conflicts of interest

Franco Frati, Ilaria Dell'Albani, Valentina Natoli and Paola Puccinelli are employees of Stallergenes Italy. Silvia Scurati is an employee of Stallergenes SA France. Cristoforo Incorvaia is a scientific consultant for Stallergenes Italy. The other authors don't have any conflicts of interest that are directly relevant to the content of the study.

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