Review Result Report

May 24, 2016

Evaluation and Licensing Division, Pharmaceutical Safety and Environmental Health Bureau

[Brand name]	Pola Wrinkle Shot Medical Serum
[Name of active ingredient]	Sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate
[Applicant]	Pola Chemical Industries, Inc.
[Date of application]	June 23, 2009

[Review results]

In the meeting held on May 20, 2016, the Committee on Cosmetics and Quasi-Drugs concluded that the product may be approved and that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

[Approval Condition]

Post-marketing surveillance on safety should be conducted for at least 2 years after approval.

Review Report

April 28, 2016
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following quasi-drug for which an approval application was submitted are as shown below.

Notice

[Brand name] Pola Wrinkle Shot Medical Serum

[Active ingredient] Sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate

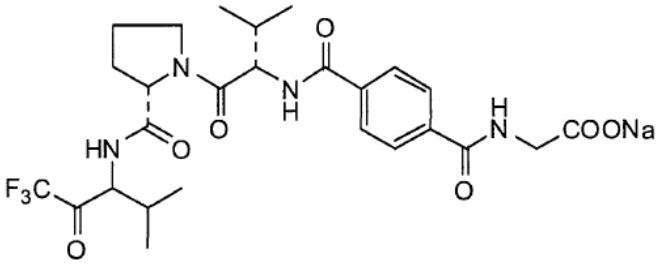
[Applicant] Pola Chemical Industries, Inc.

[Date of application] June 23, 2009

[Dosage form/Strength] Cream containing [redacted] g of sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate per 100 g

[Application classification] Quasi-drug category 1

[Chemical structure]



Molecular formula: $C_{26}H_{32}F_3N_4NaO_7$

Molecular weight: 592.54

Chemical name: Sodium 2-[4-[[[(S)-1-[[(S)-2-[(RS)-3,3,3-trifluoro-1-isopropyl-2-oxopropyl]aminocarbonyl]pyrrolidine-1-yl]carbonyl]-2-methylpropyl]aminocarbonyl]benzoylamino] acetate

[Items Warranting Special Mention] None

[Reviewing Office] Office of OTC/Quasi-drugs

Results of Review

April 28, 2016

[Brand name]	Pola Wrinkle Shot Medical Serum
[Active ingredient]	Sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate
[Applicant name]	Pola Chemical Industries, Inc.
[Date of application]	June 23, 2009
[Review results]	As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved as a quasi-drug for the following indications, and dosage and administration.
[Indications]	Improves wrinkles. Keeps the skin healthy. Protects the skin. Prevents dryness of the skin. Note) The underlined part indicates the indications related to the active ingredient of the product.
[Dosage and administration]	Take an appropriate amount and apply it to the skin.
[Approval Condition]	Post-marketing surveillance on safety should be conducted for at least 2 years after approval.

Review Report

April 28, 2016

1. Product Submitted for Registration

[Brand name]	Pola Wrinkle Shot Medical Serum
[Active ingredient]	Sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate
[Applicant name]	Pola Chemical Industries, Inc.
[Date of application]	June 23, 2009
[Dosage form/Strength]	Cream containing [redacted] g of sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate per 100 g
[Proposed indication]	Suppresses the degradation of elastin and collagen and prevents and improves wrinkles caused by ultraviolet rays. Keeps the skin healthy. Protects the skin. Prevents dryness of the skin. Note) The underlined part indicates the indications related to the active ingredient of the product.
[Proposed dosage and administration]	Take an appropriate amount and apply it to the skin.

2. Summary of the Submitted Data and Outline of Review by the Pharmaceuticals and Medical Devices Agency

A summary of the data submitted by the applicant in this application and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA) Administrative Rule No. 8/2008 dated December 25, 2008).

A. Origin or history of discovery, Use in foreign countries, and other information

The product is a medicated cosmetic/cream product containing sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate, a new active ingredient (hereinafter referred to as "the ingredient").

It is known that wrinkles are formed after the degradation of the extracellular matrices (ECM) such as elastin and collagen that constitute the dermis due to the deterioration of the function of cells and tissues and environmental factors such as ultraviolet rays and dryness, followed by repetitive movements of the facial muscles, etc.[1] In particular, exposure to ultraviolet rays results in the infiltration of neutrophils into

[1] Takema Y.*et al*, *Exp Dermal*, 5(3):145-149,1996

the dermis and the increased activity of neutrophil elastase, matrix metalloproteinase (MMP)-1, and MMP-2, which are ECM degradation enzymes.[2,3,4] Neutrophil elastase is suggested to be involved in the activation of MMP-1 and MMP-2.[4] The ingredient is considered to inhibit neutrophil elastase and indirectly suppress the activation of MMP-1 and MMP-2, thereby preventing excessive degradation of elastin and collagen.

As of July 2015, there are no drugs or cosmetics containing the ingredient in and outside Japan.

B. Physicochemical properties and specifications and testing methods

Summary of the submitted data

(1) The ingredient

1) Characterization

The ingredient is a white to reddish white powder.

The structure of the ingredient is supported by nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR), infrared spectrophotometry (IR), ultraviolet spectroscopy (UV), and mass spectroscopy (DI-FAB method).

2) Control

The proposed specifications for the ingredient include content, description, identification ([redacted]), [redacted], purity ([redacted]), [redacted], isomer ratio [[redacted]], and assay ([redacted]).

(2) Quasi-drug product

1) Formulation development

The product is a cream containing [redacted] g of sodium trifluoro-isopropyloxopropyl aminocarbonyl pyrrolidinyl carbonyl-methylpropyl aminocarbonyl benzoylamino acetate per 100 g. The product contains the following excipients: Silicic anhydride, methylpolysiloxane, sericite, glyceryl tri(2-ethylhexanoate), cross-linked methylpolysiloxane, and methacrylic ester resin powder.

2) Control

The proposed specifications for the product include content, description, identification ([redacted]), [redacted], and assay ([redacted]).

Outline of the review by PMDA

PMDA reviewed the submitted data and the responses to the inquiries and concluded that the specification items for the ingredient and the product were appropriately set.

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[2] Inomata S.*et al*, *J.Invest.Dermatol*, 120(1):128-134,2003
[3] Fisher G.J.*et al*, *Nature*, 379(6563):335-339,1996
[4] Takeuchi H.*et al*, *J Dermatol Sci*, 60(3):151-158,2010

C. Stability

Summary of the submitted data

(1) Stability of the ingredient

Stability tests of the ingredient were conducted as shown in Table 1.

Table 1 Stability tests of the ingredient

Study title	Reference batch	Storage conditions	Storage form	Storage period
Long-term test	3 batches on [redacted] or [redacted] kg scale	25°C, 60% RH	Put the sample in an inner bag made of [redacted], put it in a bag made of [redacted] together with [redacted], and seal it.	36 months
Accelerated test		40°C, 75% RH		6 months
Stress test	1 batch on [redacted] kg scale	[redacted]°C	Clear and colorless vial (airtight)	[redacted] months
		[redacted]°C, [redacted]% RH	Clear and colorless vial (open)	[redacted] months
		40°C, 75% RH		
		25°C, 1.2 million lux•hr, near-ultraviolet 200 W•hr/m^2	Clear and colorless vial (airtight)	-
			Clear and colorless vial (airtight), overall covered with aluminum foil	

In the long term test and accelerated test, [redacted] slightly decreased, but no time-course change was observed in other test items. Among the stress tests, a marked change in the description from powder to [redacted] and an increase in related substances ([redacted]% to [redacted]%, [redacted]%) were observed in the high-humidity stress test, and a decrease in the content ([redacted]% to [redacted]%) and an increase in related substances ([redacted]% to [redacted]%) were observed in the light stress test; therefore, the ingredient was considered to be affected by [redacted] and [redacted].

Based on the above, the ingredient was determined to be stable for 36 months when stored at room temperature in [redacted] and [redacted].

(2) Stability of the product

Stability tests of the product were conducted as shown in Table 2.

Table 2 Stability tests of the product

Study title	Reference batch	Storage conditions	Storage form	Storage period
Long-term test	3 batches on [redacted] kg scale	25°C, 60% RH	[redacted] container*	36 months
Accelerated test		40°C, 75% RH		6 months
Stress test	1 batch on [redacted] kg scale	[redacted]°C		[redacted] months
		[redacted]°C, [redacted]% RH	[redacted] container (open bottom)	[redacted] months
		40°C, 75% RH		
		25°C, 1.2 million lux•hr, near-ultraviolet 200 W•hr/m^2	Clear and colorless vial (airtight)	-
			Clear and colorless vial (airtight), overall covered with aluminum foil	

* [redacted] tube

In the long-term test, no time-course change was observed. In the accelerated test, a slight decrease in the content was observed, but no time-course change was observed in other test items. In the light stress test among the stress tests, a decrease in the content ([redacted]% to [redacted]%) was observed; therefore, the product was considered to be affected by light.

Based on the above, the shelf life of the product was determined to be 36 months when stored in a light-resistant container at room temperature.

Outline of the review by PMDA

PMDA reviewed the submitted data and the responses to the inquiries and concluded that the quality of the ingredient and the product was appropriately controlled.

D. Safety

Summary of the submitted data

As the safety data of the ingredient, the results of the following studies were submitted: A single-dose toxicity study (oral), repeated-dose toxicity studies (subcutaneous, intravenous), reproductive and developmental toxicity studies, antigenicity studies (skin sensitization test), genotoxicity studies (reverse mutation test, chromosomal aberration test, and bone-marrow micronucleus test), local tolerance studies (primary skin irritancy test, continuous skin irritancy test, eye irritancy test, human patch test), pharmacokinetic studies (absorption, distribution, metabolism, excretion), and a repeated subcutaneous dose toxicity study of optical isomers. As the safety data of the product, the results of the following studies were submitted: Antigenicity studies (skin sensitization test, human repeated insult patch test, and patch test after long-term use in humans), local tolerance studies (primary skin irritancy test, continuous skin irritancy test, eye irritancy test, human patch test), pharmacokinetic studies (absorption, distribution, excretion), and a continuous use study in humans. Photosensitization and phototoxicity studies were omitted for the ingredient because the molar extinction coefficient at 290 to 700 nm did not exceed 1000 $Lmol^{-1}cm^{-1}$.

(1) Single-dose toxicity

1) Single oral dose toxicity study in rats (the ingredient): Attachment D-1-1-(1)

Rats (CD [SD], 5 male and 5 female animals each per group) were given a single oral dose of the ingredient (0 and 2000 mg/kg; vehicle, physiological saline) and observed for 14 days. There were no deaths, abnormalities in clinical findings or changes in body weight. Necropsy revealed no abnormal findings in any of the animals. Based on the above, the approximate lethal dose was determined to be >2000 mg/kg.

(2) Repeated-dose toxicity

1) 4-week repeated subcutaneous dose toxicity study in rats (the ingredient): Attachment D-1-2-(1)

Rats (CD [SD], 12 male and 12 female animals each per group) were given subcutaneous doses of the ingredient (0, 32, 100, and 320 mg/kg; vehicle, physiological saline) once daily for 4 weeks. In addition, 6 male and 6 female rats (CD [SD]) per group were used as a satellite group, and plasma concentrations

were measured on the first and last days of once-daily administration of the ingredient (32, 100, and 320 mg/kg) in 3 animals each.

No animal death was observed, and no abnormalities considered to be related to administration of the ingredient were observed in clinical findings, food consumption, female estrous cycle, ophthalmology, body weight change, urinalysis, hematology, or blood biochemistry.

The measurement of plasma concentrations of the ingredient showed that the mean plasma concentrations at 1 hour post-dose were almost proportional to the dose in both males and females, and the plasma concentrations at 24 hours post-dose were below the detection limit (SSS[5] form, 0.29 μg/mL; SSR[6] form, 0.21 μg/mL) both on the first and last administration days.

Based on the above, the no-observed-adverse-effect level (NOAEL) of the ingredient was determined to be 320 mg/kg/day in both males and females.

2) 4-week repeated intravenous dose toxicity study in rats (the ingredient): Attachment D-1-2-(2)

Rats (CD [SD], 10 male and 10 female animals each per group) were given intravenous doses of the ingredient (0, 320, 1000, and 2000 mg/kg; vehicle, physiological saline) via the caudal vein once daily for 4 weeks.

There were no deaths. In the 2000 mg/kg group, the following findings were observed: Salivation during administration and decreased activity and bradypnea immediately after administration (clinical findings), increased kidney weight (organ weight), vacuolation of the proximal tubular epithelium of the kidney (histopathological examination), and increased urine volume and decreased osmolarity in males and increased total sodium excretion in males and females (urinalysis). Histopathology of the tail, the administration site, revealed vascular intimal proliferation in almost all animals in the ingredient group. This change was localized to the administration site and was attributed to vascular stimulation by the dosing solution.

Based on the above, the NOAEL of the ingredient was determined to be 1000 mg/kg/day in both males and females, except for effects on the administration site.

3) 13-week repeated subcutaneous dose toxicity study with 4 weeks of recovery in rats (the ingredient): Attachment D-1-2-(3)

Rats (CD [SD], 12 male and 12 female animals each per group) were given subcutaneous doses of the ingredient (0, 100, 320, and 1000 mg/kg; vehicle, physiological saline) on the back once daily for 13 weeks. In the 0 and 1000 mg/kg groups, 6 males and 6 females each were added to make the recovery group, for which a 4-week recovery study was conducted after completion of 13-week administration. In addition, other 4 male and 4 female rats (CD [SD]) per group were used as a satellite group, and plasma concentrations were measured on the first and last days of once-daily administration of the ingredient (32, 100, 320, and 1000 mg/kg).

No animal death was observed, and no abnormalities considered to be related to administration of the ingredient were observed in food consumption, body weight change, or ophthalmology.

Changes at the administration site were observed as dark red spots on the subcutis at necropsy in the

[5] Sodium2-[4-[[(*S*)-1-[[(*S*)-2-[[(*S*)-3,3,3-trifluoro-l-idopropyl-2-oxopropyl]aminocarbonyl]benzoylamino]acetate
[6] Sodium2-[4-[[(*S*)-1-[[(*S*)-2-[[(*R*)-3,3,3-trifluoro-l-idopropyl-2-oxopropyl]aminocarbonyl]benzoylamino]acetate

≥320 mg/kg groups, crust in the 1000 mg/kg group, and hemorrhage and inflammatory changes at the pathological examination. However, these changes tended to recover during the recovery study, suggesting that the local effects were mild.

Urinalysis showed increased sodium excretion in the 1000 mg/kg group, which was not associated with changes in serum electrolytes. Hematology showed decreased hemoglobin, hematocrit, and/or red blood cell count and increased reticulocyte and platelet counts in the 1000 mg/kg group. All of these changes were mild and likely to be secondary changes attributable to hemorrhage under the administration site. A blood biochemistry test showed decreased albumin, total protein, albumin/globulin ratio, and glucose, and increased α_2- and β-globulin, but these changes were slight. In addition, a male animal in the 1000 mg/kg group had decreased absolute and relative weights of the liver and an increased relative weight of the kidney, which were slight changes without histological changes. Therefore, these changes were not considered toxicologically significant. In the recovery study, these changes were resolved, showing favorable recovery.

When plasma concentrations of the ingredient were measured, plasma concentrations of both SSS[5] and SSR[6] forms increased dose-dependently. When males and females were compared, the concentrations of both SSS and SSR forms tended to be higher in males. When SSS and SSR forms were compared, the concentrations of SSR form tended to be similar or lower in both males and females in each group. As the effects of repeated administration, the concentrations of both SSS and SSR forms tended to be high on the last day of administration in each group, but the plasma concentrations at 24 hours post-dose on the first and last days of administration were below the lower limit of quantitation (SSS form, 0.26 μg/mL; SSR form, 0.25 μg/mL).

Based on the above, the NOAEL of the ingredient was determined to be 1000 mg/kg/day, except for effects on the administration site.

(3) Reproductive and developmental toxicity

1) Subcutaneous dose study in rats before pregnancy and in early pregnancy (the ingredient): Attachment D-1-3-(1)

Rats (CD [SD], 23 males and 23 females each per group) were given subcutaneous doses of the ingredient (0, 32, 100, and 320 mg/kg; vehicle, physiological saline) on the back once daily for 9 weeks before the start of mating, during the mating period, and until the day before necropsy after the end of mating in males, and for 2 weeks before the start of mating, during the mating period, and until Day 7 of gestation after the establishment of mating in females.

No effects of the ingredient were observed on the clinical findings, body weight, food consumption, or necropsy in any of males or females in any of the groups of parent animals. The ingredient had no effects on the number of estruses, estrous cycle, copulation index, fertility index, or number of days required for copulation in any group. No effects of the ingredient were observed on the number of corpora lutea, number of implantations, number of embryo-fetal deaths, number of live fetuses, sex ratio, body weight of live fetuses, or placental weight in any group.

Based on the above, the NOAELs of the ingredient for general toxicity, reproductive function in parent animals, and fetuses were all determined to be 320 mg/kg/day.

2) Study on effects on pre- and post-natal development and maternal function in rats (the ingredient): Attachment D-1-3-(2)

Rats (CD [SD], 20 females after mating per group) were given subcutaneous doses of the ingredient (0, 100, 320, and 1000 mg/kg; vehicle, physiological saline) on the back once daily from Day 7 of gestation to Day 21 post partum.

In F_0 dams, no abnormal changes in body weight or food consumption were observed. There were no effects on the delivery, nursing status, gestation period, number of implantation sites, or live birth index.

In F_1 offspring, a decreased fertility index was observed in males and females in the 1000 mg/kg group. This finding was considered attributable to the atrophy of the testis in 1 male animal and not likely to be related to the ingredient, although the cause is unknown. There were no effects on survival or development of offspring.

Based on the above, the NOAELs were determined to be 1000 mg/kg/day for general toxicity and reproductive toxicity in dams, and 320 mg/kg/day for F_1 pups.

3) Subcutaneous dose study during the period of fetal organogenesis in rats (the ingredient): Attachment D-1-3-(3)

Rats (CD [SD], 24 females after mating per group) were given subcutaneous doses of the ingredient (0, 100, 320, and 1000 mg/kg; vehicle, physiological saline) on the back once daily from Days 7 to 17 of gestation.

In dams, no deaths occurred, and no effects of the ingredient were observed on the clinical findings, body weight, food consumption, or necropsy.

In fetuses, examination at cesarean section revealed no effects on the number of dead embryos/fetuses, body weight of live fetuses, etc., and no embryonic lethality or suppression of fetal development was observed. The morphological examination of the external surface, internal organs, and skeleton revealed no effects of the ingredient.

Based on the above, the NOAELs of the ingredient in dams and fetuses were both determined to be 1000 mg/kg/day.

4) Subcutaneous dose study during the period of fetal organogenesis in rabbits (the ingredient): Attachment D-1-3-(4)

Rabbits (NZW, 15 females after mating per group) were given subcutaneous doses of the ingredient (0, 10, 100, and 1000 mg/kg; vehicle, physiological saline) on the back once daily from Days 6 to 18 of gestation.

In dams, no deaths occurred, and decreased food consumption without body weight change was observed in the ≥100 mg/kg dose groups, and abortion was observed in 1 animal with a loss of appetite in the 1000 mg/kg group. There were no changes in clinical findings in any group, nor were there any effects of the ingredient on necropsy at cesarean section.

In fetuses, examination at cesarean section revealed no effects on the number of dead embryos/fetuses, body weight of live fetuses, etc., and no embryonic lethality or suppression of fetal development was observed. The morphological examination of the external surface, internal organs, and skeleton revealed no effects of the ingredient.

Based on the above, the NOAELs of the ingredient in dams and fetuses were both determined to be 1000 mg/kg/day.

(4) Antigenicity

1) Skin sensitization test (maximization test) in guinea pigs (the ingredient): Attachment D-1-4-(1), (3)

The test was conducted in guinea pigs (Hartley, 6 males per group) according to the maximization test with the concentrations of the ingredient as follows: Sensitization, [redacted]% (challenge: [redacted]%, [redacted]%, [redacted]%); sensitization, 10% (challenge: [redacted]%, [redacted]%, 10%); and sensitization, 30% (challenge: [redacted]%, 10%, 30%). No skin reactions were observed at any challenge concentrations in the [redacted]% sensitization group. In the 10% sensitization group, no skin reactions were observed at challenge concentrations of ≤[redacted]%, but skin reactions were observed at 10% challenge sites, and the positive rate was 100%. In the 30% sensitization group, skin reactions were observed at all challenge concentrations, and the positive rate was 100% for all.

When the test was conducted in the 5% sensitization group, no skin reactions were observed at any challenge concentrations ([redacted]%, [redacted]%, 5%).

Based on these results, it was concluded that although the ingredient has sensitization potential, no skin sensitization may occur at concentrations of ≤5%.

2) Skin sensitization test (Buehler test) in guinea pigs (the ingredient): Attachment D-1-4-(2)

The test was conducted in guinea pigs (Hartley, 6 males per group) according to the Buehler test with the concentrations of the ingredient as follows: Sensitization, 10% (challenge: 10%); and sensitization, 30% (challenge: 30%). No skin reactions were observed, and it was concluded that the ingredient has no skin sensitization potential.

3) Sensitization test (local lymph node assay) in mice (the ingredient): Attachment D-1-4-(4)

The test was conducted in mice (CBA/J, 5 females per group) according to the local lymph node assay (concentration of the ingredient: 10%, 25%, 50%). The stimulation indices (SIs) were respectively 1.2, 1.1, and 1.6, from the low-concentration group, with all SIs below 3.0, the reference value for positivity. Therefore, the ingredient was determined to be negative for sensitization.

4) Skin sensitization test (adjuvant and patch test) in guinea pigs (preparation): Attachment D-2-1-(1)

The test was conducted in guinea pigs (Hartley, 6 males per group) according to the adjuvant and patch test (test substances:The product, preparation containing [redacted]% the ingredient,[7] base). No skin reactions were observed, and it was concluded that the product has no skin sensitization potential.

5) Human repeated insult patch test (HRIPT) (preparation): Attachment D-2-1-(2)

A human repeated insult patch test (HRIPT) was performed in healthy Japanese women (55

[7] A preparation using the same base as the product with the content of the ingredient increased by [redacted]% and the content of some bases reduced by the same amount

participants) using a preparation containing [redacted]% the ingredient.[7] In the test, sensitization was induced by the application of the preparation containing [redacted]% the ingredient[7] as closed patches (0.05 mL/cm^2) on the dorsal skin for 3 consecutive weeks (48 hours × 3 times/week, 9 times in total). Subsequently, the participants were given a rest for 2 weeks, followed by a challenge application (48-hour closed patch of the preparation containing [redacted]% the ingredient at the application site for sensitization and a new site [upper arm], 0.05 mL/cm^2). Skin reactions were assessed at 2 or 24 hours after removal of the patch during the sensitization phase and at 2 and 48 hours after removal of the patch during the challenge phase.

No skin reactions were observed in 54 of the 55 participants, excluding 1 participant withdrawn for personal reasons, on any of the assessment days during the sensitization phase or the challenge phase. Based on the above, the product was considered unlikely to induce skin sensitization.

6) Patch test after long-term use in humans (preparation): Attachment D-2-1-(3)

A patch test was conducted using the ingredient [redacted]%[8] solution and the product after long-term use of the product (applied to the face twice daily for 24 weeks) in healthy Japanese women (110 participants). During the use period, skin observation and interview by a dermatologist were performed at baseline, Week 4, Week 12, and Week 24.

Of a total of 110 participants, adverse events were reported in 2 participants (mild or slight redness and itching in both) during the use period in 102 participants excluding 8 participants withdrawn (7 for personal reasons and 1 who met an exclusion criterion). All the events were determined to be unrelated to the product.

In the patch test, approximately 0.015 mL of a sample was applied as closed patches using Finn Chamber for 48 hours, and assessment was performed by a dermatologist according to the International Contact Dermatitis Research Group (ICDRG) criteria[9] at 48 hours (1.5 to 2 hours after removal of the patch), 72 hours, and 1 week after the application. The results showed no positive reaction of (+) or greater. Erythema only (+?) was observed in some participants (1 participant with the ingredient [redacted]% solution, 1 participant with the product, 1 participant with the placebo, 1 participant with the vehicle control [water for injection], and 1 participant with the negative control [physiological saline]) at 48 hours after the application, but the reactions to the ingredient [redacted]% solution and the product had disappeared at 72 hours after the application. Based on the above, the product was considered unlikely to cause skin irritation or sensitization.

(5) Genotoxicity (the ingredient): Attachment D-1-5-(1), (2), (3)

For the ingredient, a reverse mutation test in bacteria, a chromosomal aberration test in Chinese hamster lung (CHL/IU) cell line, and a bone-marrow micronucleus test in mice were conducted. In the chromosomal aberration test, the ingredient at a dose of 5000 μg/mL induced structural aberrations at a rate of 6.0% in the short-term treatment without S9 mix, but no reproducibility was found in the confirmatory test; therefore, the chromosomal aberration inducibility of the ingredient was determined to be negative. The other tests were negative.

[8] A concentration 10 times higher than the concentration of the ingredient in the product was selected to detect weak sensitization.
[9] (−), no reaction; (+?), erythema only; (+), erythema + infiltration, papule; (++), erythema + infiltration + papule, vesicles; (+++), coalescent vesicles; IR, irritation reaction; NT, not performed (+ or greater is defined as positive reaction)

(6) Local tolerance

1) Primary skin irritancy test in rabbits (the ingredient): Attachment D-1-6-(1)

Lint cloth soaked with 0.5 mL of the ingredient ([redacted]%, 10%, 30%[10]) was applied to the dorsal skin of rabbits (NZW, 6 males per group) as closed patches for 24 hours. Assessment was performed 24, 48, and 72 hours after the application according to the Draize criteria, showing no skin reactions at any application sites.

2) Continuous skin irritancy test in guinea pigs (the ingredient): Attachment D-1-6-(2)

To the dorsal skin of guinea pigs (Hartley, 6 males per group), 0.01 mL of the ingredient ([redacted]%, 10%, 30%[10]) was openly applied once daily for 14 days. Assessment was performed daily according to the Draize criteria, showing no skin reactions in any of the animals throughout the observation period.

3) Eye irritancy test in rabbits (the ingredient): Attachment D-1-6-(3)

In the conjunctival sac of rabbits (NZW, 6 males per group), 0.1 mL of the ingredient ([redacted]%, 10%, 30%[10]) was instilled once (eyes not washed after the instillation in 3 rabbits, eyes washed after the instillation in 3 rabbits). Assessment was performed at 1, 24, 48, 72, and 96 hours after instillation according to the Draize criteria, showing no irritation reactions in any of the animals.

4) Human patch test (the ingredient): Attachment D-1-6-(4)

Approximately 0.015 mL of an aqueous solution of the ingredient ([redacted]%, [redacted]%, [redacted]%[11]) and approximately 0.015 g of petrolatum dispersion ([redacted]%, [redacted]%, [redacted]%[11]) were applied as closed patches using Finn Chamber for 24 hours in 49 healthy Japanese adults. Assessment was performed at 24 hours (30 minutes after patch removal) and 48 hours after the application according to the Japanese criteria.[12] Mild erythema (±) was observed in some participants (2 participants with the ingredient [redacted]% solution, 1 participant with [redacted]% solution, 6 participants with [redacted]% solution, 10 participants with the vehicle control [water for injection], and 2 participants with the vehicle control [petrolatum]) at 24 hours after the application, and (+) positive reaction in 1 participant with the vehicle control (petrolatum). These reactions had disappeared at 48 hours after the application. Based on the above, the primary skin irritancy caused by the ingredient was considered to be weak.

5) Primary skin irritancy test in rabbits (preparation): Attachment D-2-2-(1)

Lint cloth soaked with 0.5 mL each of the product, a preparation containing [redacted]% the ingredient,[7] and base was applied to the dorsal skin of rabbits (NZW, 6 males per group) as closed patches for 24 hours. Assessment was performed 24, 48, and 72 hours after the application according to the Draize criteria. Very mild erythema (score 1) was observed in all animals at 24 hours after the application, but all of them had disappeared at 72 hours after the application. Based on the above, the product was considered to have mild primary skin irritancy.

[10] Maximum soluble dose in vehicle (physiological saline)

[11] A concentration 20 times higher than the concentration of the ingredient in the product was selected to detect weak sensitization.

[12] (−), no reaction; (±), mild erythema; (+), erythema; (++), erythema + edema; (+++), erythema + edema + papule or vesicles; (++++), coalescent vesicles (T Sugai. Contact dermatitis and patch test. Skin research. 19:210,1977 [in Japanese])

6) Continuous skin irritancy test in guinea pigs (preparation): Attachment D-2-2-(2)

To the dorsal skin of guinea pigs (Hartley, 6 males per group), 0.01 mL each of the product, a preparation containing [redacted]% the ingredient,[7] and base was openly applied once daily for 14 days. Assessment was performed daily according to the Draize criteria, showing no skin reactions in any of the animals throughout the observation period. Based on the above, the product was considered to have no continuous skin irritancy.

7) Eye irritancy test in rabbits (preparation): Attachment D-2-2-(3)

In the conjunctival sac of rabbits (NZW, 6 males per group), 0.1 mL each of the product, a preparation containing [redacted]% the ingredient,[7] and base was instilled once (eyes not washed after the instillation in 3 rabbits, eyes washed after the instillation in 3 rabbits). Assessment was performed at 1, 24, 48, 72, and 96 hours after instillation according to the Draize criteria, showing no irritation reactions in any of the animals. Based on the above, the product was considered to have no eye irritancy.

8) Human patch test (preparation): Attachment D-1-6-(4)

Approximately 0.015 g each of the product, a preparation containing [redacted]% the ingredient,[7] and base was applied as closed patches using Finn Chamber for 24 hours in 49 healthy Japanese adults. Assessment was performed at 24 hours (30 minutes after patch removal) and 48 hours after the application according to the Japanese criteria.[8] Mild erythema (±) was observed in some participants (1 participant with the product and 2 participants with base) at 24 hours after the application, but these reactions had disappeared at 48 hours after the application. Based on the above, the primary skin irritancy caused by the product was considered to be weak.

(7) Absorption, distribution, and excretion

1) Absorption (the ingredient): Attachment D-1-7-(1)

Following a single intravenous dose of 10 mg/kg of the ingredient labeled with ^{14}C in rats (SD, 3 males), the plasma radioactivity concentration peaked at 15277 ng eq./mL at 5 minutes post-dose and decreased with a half-life of 11 minutes until 1 hour post-dose. It then decreased at a slower rate than that up to 1 hour post-dose and fell below the detection limit (28 ng eq./mL) after 6 hours post-dose. The area under the radioactivity concentration-time curve (AUC) up to 4 hours post-dose was 4.84 μg eq.•hr/mL.

2) Distribution (the ingredient): Attachment D-1-7-(1)

Following a single intravenous dose of 10 mg/kg of the ingredient labeled with ^{14}C in rats (SD, 3 males including 1 male for autoradiography), the radioactivity concentration was 2.5 to 3.5 times higher in the kidney, bladder, and liver than in plasma (10200 ng eq./mL) at 5 minutes post-dose, but was lower in other tissues than in plasma. At 8 hours post-dose, radioactivity was detected only in the large intestine, kidney, and liver, and was below the detection limit in other tissues and plasma. At 72 hours post-dose, radioactivity was detected only in the kidney at 263 ng eq./g, which accounted for about 0.7% of that at 5 minutes post-dose.

In whole-body autoradiography, high radioactivity was detected in urine in the bladder, intestinal contents, and stomach contents at 5 minutes post-dose, followed by the kidney and liver. No radioactivity

was detected in any tissues at 72 hours post-dose.

3) Metabolism (the ingredient): Attachment D-1-7-(2), (3)

Following a single intravenous dose of 10 mg/kg of the ingredient labeled with ^{14}C in rats (SD, 1 male), the metabolites in urine, bile, and feces were searched for by thin-layer chromatography. In urine, bile, and feces, the unchanged compound was the most abundant radioactive substance, accounting for 95.08%, 93.07%, and 60.11% of the total radioactivity in the samples, respectively. In feces, 1 metabolite accounted for 29.85% following the unchanged compound. Since this metabolite was hardly detected in bile, it was considered to be produced in the gastrointestinal tract.

To rat dorsal skin homogenate (Crl: CD [SD], 10 males), the ingredient was added to make 20 μg/mL, and the optical conversion and metabolism were evaluated. The residual ratio of SSS[5] and SSR[6] forms of the ingredient and the ratio of SSS form in the ingredient remained almost unchanged until 24 hours after the start of reaction regardless of the addition of skin homogenate.

Based on the above, the ingredient was considered unlikely to be metabolized or optically converted in the body or skin.

4) Excretion (the ingredient): Attachment D-1-7-(1)

Following a single intravenous dose of 10 mg/kg of the ingredient labeled with ^{14}C in rats (SD, 3 males), 16.4%, 16.6%, and 16.7% of the dose were excreted by 4, 24, and 120 hours post-dose, respectively, in urine; and 78.1% and 80.8% of the dose were excreted by 24 and 120 hours post-dose, respectively, in feces. The body retention rate at 120 hours post-dose was 0.0% of the dose. Approximately 95% of the administered radioactivity was excreted in urine and feces by 24 hours post-dose, with the major route of excretion being feces.

Following a single intravenous dose of 10 mg/kg of the ingredient labeled with ^{14}C in bile-duct cannulated rats (SD, 3 males), 67.0%, 69.0%, and 69.7% of the dose was excreted by 1, 2, and 48 hours post-dose, respectively, in bile. Also, 26.3% and 26.8% of the dose were excreted by 4 and 48 hours post-dose, respectively, in urine; and 0.2% of the dose was excreted by 48 hours post-dose in feces. The body retention rate at 48 hours post-dose was 0.4% of the dose. Excretion of radioactivity in bile was almost complete by 1 hour post-dose.

5) Absorption (preparation): Attachment D-2-3-(1)

Following a single percutaneous dose of [redacted] to [redacted] mg/kg of a preparation containing [redacted]% the ingredient labeled with ^{14}C in rats (Crl: CD [SD], 3 males), the plasma radioactivity concentration was below the detection limit (1.21 ng eq./mL) at 2 to 168 hours post-dose.

6) Distribution (preparation): Attachment D-2-3-(2)

Following a single percutaneous dose of [redacted] to [redacted] mg/kg of a preparation containing [redacted]% the ingredient labeled with ^{14}C in rats (Crl: CD [SD], 1 male), the radioactivity concentrations in the dermis at 2, 8, and 24 hours post-dose were 1525.46, 945.26, and 321.87 ng eq./g, respectively, indicating elimination of radioactivity over time.

When distribution of radioactivity in the administration site skin was investigated by

microautoradiography, radioactivity derived from the ingredient labeled with ^{14}C was detected in the stratum corneum, epidermis, and dermis 2 hours post-dose, but almost no radioactivity was detected in the sebaceous gland, hair follicle, subcutaneous tissue, and muscle layer. Radioactivity disappeared over time at each site. At 8 and 24 hours post-dose, only slight radioactivity was detected in the stratum corneum and epidermis.

7) Excretion (preparation): Attachment D-2-3-(1)

Following a single percutaneous dose of [redacted] to [redacted] mg/kg of a preparation containing [redacted]% the ingredient labeled with ^{14}C in rats (Crl: CD [SD], 3 males), [redacted]% of the dose was excreted in urine, and [redacted]%[13] of the dose was excreted in feces by 168 hours post-dose. No radioactivity was excreted in expired air. In the absorbent cotton used for wiping at 24 hours post-dose, [redacted]% of the administered radioactivity was detected, and in the animal jacket at 168 hours post-dose, [redacted]% was detected. In the administration site skin isolated at 168 hours post-dose, [redacted]% of the administered radioactivity was detected, and in the carcasses other than the administration site skin, [redacted]% was detected. The percutaneous absorption of the product was estimated to be [redacted]% from the sum of the urinary, fecal, and expiratory excretion rates and the body retention rate.

(8) Repeated subcutaneous dose toxicity study of optical isomers in rats: Attachment D-1-8-(1)

To rats (CD [SD], 5 males and 5 females each per group), an optical isomer of the ingredient (SSS[5] form or SSR[6] form) was subcutaneously administered at a dose of 100, 320, or 1000 mg/kg/day once daily for 2 weeks.

Although findings related to inflammation were obeserved at the administration site, no clear systemic toxicity was found. When SSS and SSR forms were compared, similar changes were found with both forms, but in the local changes, there were differences in the proliferation of fibroblasts, the frequency of bleeding and inflammatory cell infiltration, and the frequency and intensity of degeneration/necrosis and ulcer in the subcutaneous tissues, and the toxic effects tended to be stronger with SSR form than with SSS form.

Based on the above, in terms of the local effect (under the skin at the administration site), the NOAEL of SSS form was considered to be 100 mg/kg/day, and that of SSR form was estimated to be less than 100 mg/kg/day. In terms of the systemic toxicity, no obvious toxicity differences were found between the SSS and SSR form groups, and the NOAEL was determined to be 1000 mg/kg/day for both forms under conditions in this study.

(9) Continuous use study in humans: Attachment D-2-4-(1) (the product)

A 24-week continuous use study of the product was conducted in 52 healthy Japanese women to evaluate the safety of the product. The participants applied the product twice daily (morning and evening) to the parts of the face where wrinkles form (predetermined by the participant, including the skin around eyes and mouth). A dermatologist observed the skin of the participants on the starting day, and at Weeks 4, 12, and 24.

In 51 out of the 52 participants in total, excluding 1 participant who withdrew for personal reasons, no

[13] [redacted]±[redacted] (mean±standard deviation) %

worsening of symptoms was observed in each skin observation item of dryness, desquamation, pruritus/scratch, erythema, and papule as compared to the study start date. An adverse event was reported in 1 participant (a slight half-rice-grain–sized papule), which was determined to be unrelated to the product. No adverse reactions were reported in any participant. Based on the above, it was concluded that the product could be used safely.

Outline of the review by PMDA

Based on the submitted data, PMDA discussed the safety of the ingredient and the product at the Expert Discussion, including the following points.

PMDA has concluded that there is no particular problem with the safety of the product when used in humans. However, taking account of the comments from the expert advisors, PMDA has concluded that, because the product is a quasi-drug containing a new active ingredient and its use experience in humans is limited, and it has a slight risk of sensitization or irritation although it is considered unlikely that it would cause skin sensitization in actual use, it is necessary to advise users to avoid excessive use and adequately caution about these risks after marketing, and to conduct a post-marketing surveillance to collect the information on safety and proper use in actual use after the scope of users is expanded to general consumers.

(1) Safety of the ingredient and the product

Regarding safety of the ingredient, the NOAEL was determined to be 1000 mg/kg/day in the 13-week repeated subcutaneous dose toxicity study, and the lowest NOAEL was determined to be 320 mg/kg/day in the reproductive and developmental toxicity study (subcutaneous administration), a study on effects on pre- and post-natal development and maternal function. The safety factors calculated from the percutaneous absorption amount estimated in actual use of the product were 2777778 and 888889 times, respectively. In the local tolerance studies, the following local reactions were observed, but there were no particular problems. In the repeated subcutaneous dose toxicity study using optical isomers, histopathological damages were found at the local subcutaneous site of administration, but it was considered very unlikely that the same damage would occur in actual use based on the percutaneous absorption amount of the ingredient. In addition, in the clinical trial using the product, no adverse events were reported in any participant throughout the use period. Based on the above, PMDA has concluded that there is no particular problem with the safety of the product in use.

- In the primary skin irritancy test using the product, very mild erythema was observed in all animals 24 hours after application but disappeared in all animals 72 hours after application. In the 14-day continuous skin irritancy test, no skin reactions were observed in any animal.
- In the human patch test using the ingredient, mild erythema (the ingredient [redacted]%, [redacted]%, [redacted]% solution: 6/49 participants, 1/49 participants, 2/49 participants) was observed 24 hours after application. However, the number of participants with erythema was smaller than that with the vehicle control, water for injection (10/49 participants), and all reactions had disappeared at 48 hours after application. In the human patch test using the product, mild erythema (1/49 participants) was also observed, so was with the base (2/49 participants), and all reactions had disappeared at 48 hours after application.

For the ingredient, among the nonclinical sensitization tests, negative results were shown in 2 tests (Buehler test and adjuvant and patch test), but in the skin sensitization test in guinea pigs (maximization test), the risk of skin sensitization was suggested at a concentration exceeding [redacted]%. Therefore, the expert advisors pointed out that further investigation of skin sensitization was desirable. In response to this suggestion, the applicant conducted an additional sensitization test in mice (local lymph node assay), and the results demonstrated that the ingredient was negative for sensitization at a concentration up to [redacted] times the concentration of the ingredient in the product ([redacted]%). Also, in the human patch test after long-term use (the product and the ingredient [redacted]% solution [10 times the concentration of the ingredient in the product]), no skin sensitization was observed (see D. Safety, (4) Antigenicity, 3) and 6)).

In addition to the results of these additional studies, the 24-week continuous use study of the product in humans and the clinical trial (see E. Indications, (2) Clinical trial) did not show skin sensitization; therefore, PMDA has concluded that the ingredient is unlikely to cause skin sensitization in actual use.

In addition, the expert advisors presented an opinion that, although skin sensitization is unlikely to occur in actual use in consideration of these additional study data, the users should be adequately cautioned for the reason that there is the risk of unexpected sensitization because the product may be used in humans with various skin conditions for a longer period after marketing, and the possibility of irritancy cannot be ruled out based on the results of the primary skin irritancy test and patch test.

PMDA's view on the safety of the product is as described below.

Although there is no particular concern about the overall safety of the ingredient or the product, some test results suggest a slight risk of sensitization. However, taking into account the results of the additional nonclinical studies, human patch test, human use study, etc. it is considered very unlikely that skin allergy, etc. will occur in actual use, and it is considered unnecessary to place any special restriction on its use including the administration period. Nevertheless, because the product is a quasi-drug containing a new active ingredient, the experience of use in humans at the development stage is limited, and the product will be used for a long period in humans with various skin conditions for the first time after marketing, PMDA instructed the applicant that the applicant should advise users to avoid excessive use and adequately caution about these risks, and should conduct a post-marketing surveillance to collect the information on safety and proper use in actual use after the scope of users is expanded to general consumers.

The applicant replied that they would adequately caution users based on the above and construct a system to take prompt action when any event of concern is found in post-marketing surveillance.

E. Indications

Summary of the submitted data

(1) Basic studies supporting indications

As basic studies to support the indications, the following effects of the ingredient were investigated: Inhibitory effects on human neutrophil elastase activities, inhibitory effects on other proteolytic enzyme activities, inhibitory effects on the activation of MMP-1 and MMP-2 by human neutrophil elastase, inhibitory effects on the activation of collagenase in the skin, improving effects on dermal collagen fibers, improving effects on wrinkles in a hairless mouse photoaged model, and improving effects on dermal collagen fiber bundles.

1) Inhibitory effects on human neutrophil elastase activities: Attachment E-1-1-(1)

The ingredient, SSS form of the ingredient, or SSR form of the ingredient was added to human neutrophil elastase. After [redacted], Succinyl-L-Alanyl-L-Alanyl-L-Alanine p-nitroanilide was added as the substrate, and the mixture was allowed to react at 37°C for [redacted] minutes. Then, elastase activities were determined by measuring the absorbance at 410 nm. Table 3 shows the concentration at which each test substance inhibits 50% of the activity of human neutrophil elastase (inhibitory concentration 50%: IC_{50}). The active form of the ingredient against human neutrophil elastase activities was considered to be SSS form.

Table 3 IC_{50} for neutrophil elastase activities

Test substance	IC_{50} (mol/L)	Activity ratio (relative to the ingredient)
The ingredient	2.93×10^{-7}	1
SSS form of the ingredient	1.35×10^{-7}	2.17
SSR form of the ingredient	5.89×10^{-6}	0.05

Mean (n=3)

2) Inhibitory effects on other proteolytic enzyme activities: Appendix E-1-2-(1) to (7)

The ingredient was reacted with MMP-1 (collagenase-1), MMP-2 (gelatinase A), MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-9 (gelatinase B), MMP-12 (macrophage metalloelastase), or MMP-13 (collagenase-3) at 37°C for 60 minutes. After that, the substrate[14] was added, and enzyme activities were determined by measuring the absorbance at 412 nm. Table 4 shows the IC_{50} of the ingredient on the MMP activities. The ingredient did not inhibit the enzyme activities of proteolytic enzymes MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-12, and MMP-13.

Table 4 IC_{50} for MMP activities

Enzyme name	IC_{50} (mol/L)
MMP-1	$>1.0\times10^{-2}$
MMP-2	$>1.0\times10^{-3}$
MMP-3	$>1.0\times10^{-3}$
MMP-7	$>1.0\times10^{-3}$
MMP-9	$>1.0\times10^{-3}$
MMP-12	$>1.0\times10^{-3}$
MMP-13	$>1.0\times10^{-3}$

Mean (n=2)

[14] (Ac-Pro-Leu-Gly-[(*S*)-2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OEt)

3) Inhibitory effects on the activation of MMP-1 and MMP-2 by human neutrophil elastase: Appendix E-1-3-(1), (2)

Human neutrophil elastase (final concentration: [redacted] μg/mL) and the ingredient (final concentration: 2.9×10^{-9} to 10^{-5} mol/L) were added to normal human dermal fibroblasts, and the fibroblasts were cultured for 25 hours. Then, the supernatants were assayed by immunoblotting, and the intensities of the bands of pro- and active MMP-1 were determined by densitometric analysis. The proportion of active MMP-1 to all MMP-1 is shown in Table 5, suggesting that the ingredient inhibits the activation of MMP-1 by human neutrophil elastase.

Table 5 Proportion of active MMP-1

Concentration of the ingredient (mol/L)	Proportion of active form (%)
0	42.40±3.40
2.9×10^{-9}	34.04±3.61
2.9×10^{-8}	16.80±1.14
2.9×10^{-7}	13.60±1.12
2.9×10^{-6}	4.31±1.23
2.9×10^{-5}	2.87±0.85

Mean±standard error (n=3)

Human neutrophil elastase (final concentration: [redacted] μg/mL) and the ingredient (final concentration: 1.0×10^{-7} to 10^{-4} mol/L) were added to normal human dermal fibroblasts, and the fibroblasts were cultured for 24 hours. Then, the supernatants were assayed by gelatin zymography, and the intensities of the bands of pro- and active MMP-2 were determined by densitometric analysis. The proportion of active MMP-2 to all MMP-2 is shown in Table 6, suggesting that the ingredient inhibits the activation of MMP-2 by human neutrophil elastase.

Table 6 Proportion of active MMP-2

Concentration of the ingredient (mol/L)	Proportion of active form (%)
0	29.00±1.22
1.0×10^{-7}	30.04±1.09
1.0×10^{-6}	29.02±2.06
1.0×10^{-5}	21.95±2.21
1.0×10^{-4}	23.03±1.79

Mean±standard deviation (n=3)

4) Inhibitory effects on the activation of collagenase in the skin: Appendix E-1-4-(4)

Hairless mice (5 females per group) were irradiated with ultraviolet rays[15] [redacted] time(s) a week for [redacted] weeks, and 100 μL of the ingredient (3.0 w/v%) solution or the vehicle control (ethanol) was administered percutaneously [redacted] time(s) a week for 1, 2, 4, or 8 weeks from the next day after completion of the irradiation period. After the end of administration, the active collagenase activity and the total collagenase activity in the protein extracted from skin were determined[16]. Table 7 shows the proportion of active collagenase to total collagenase. The ingredient was shown to inhibit the activation of collagenase in the skin.

Table 7 Proportion of active collagenase

Timing	Agent	Proprotion of active form
Week 1	The ingredient	0.68 ± 0.30
	Vehicle control	0.57 ± 0.25
Week 2	The ingredient	0.64 ± 0.10
	Vehicle control	0.64 ± 0.09
Week 4	The ingredient	0.25 ± 0.18*
	Vehicle control	0.69 ± 0.19
Week 8	The ingredient	0.15 ± 0.09
	Vehicle control	0.33 ± 0.14

Mean±standard deviation (4 or 5 animals)
(4 animals for vehicle control at Week 4 only)
*: $p<0.0125$ (vs. vehicle control, t-test)

5) Improving effects on dermal collagen fibers: Attachment E-1-4-(3)

Hairless mice (5 females per group) were irradiated with ultraviolet rays[15] [redacted] time(s) a week for [redacted] weeks, and 100 μL of the ingredient (3.0 w/v%) solution or the vehicle control (ethanol) was administered percutaneously [redacted] time(s) a week for 8 weeks from the next day after completion of the irradiation period. After completion of administration, the condition of dermal collagen fibers was observed by transmission electron microscopy (TEM) using the resin-embedded ultra-thin sections of the skin. The diameters of collagen fibers were distributed unevenly and widely between 30 and 80 nm in the vehicle control group, while they were distributed mainly around 50 to 60 nm in the ingredient group, demonstrating an improvement in uniformity.

6) Improving effects on wrinkles in hairless mouse photoaged model: Attachment E-1-4-(1), (5)

Hairless mice (5 females per group) were irradiated with ultraviolet rays[15] [redacted] time(s) a week for [redacted] weeks, and 100 μL of the ingredient ([redacted], [redacted], and [redacted] w/v%) solution, the vehicle control (ethanol), or positive control (0.05 w/v% retinoic acid and 0.05 w/v% retinol) was administered percutaneously [redacted] time(s) a week for 8 weeks from the next day after completion of the irradiation period. After completion of administration, a skin replica was prepared, and using the shaded image of the replica, the wrinkle score was evaluated according to the 6-grade wrinkle score criteria based on the standard replica, and the wrinkle length was determined by image analysis. The results are shown in Table 8.

[15] The ultraviolet irradiation dose was set to [redacted].
[16] The active collagenase activity was determined using a fluorescence plate reader (excitation at 495 nm and absorption at 520 nm) after adding fluorescent-labeled type I collagen solution to the extracted protein and reacting at 37°C for 3 hours. The total collagenase activity was determined by adding 4-aminophenylmercuric acetic acid, a collagenase activating reagent, to the extracted protein and processing in the same manner.

Table 8 Wrinkle score and wrinkle length

Agent	Wrinkle score	Wrinkle length (mm/4 cm^2)
Vehicle control	4.13±0.43	241.88±102.23
0.05 w/v% retinoic acid	1.10±0.06†	27.87±8.18††
0.05 w/v% retinol	1.75±0.67†	73.64±31.51††
[redacted] w/v% the ingredient	3.95±0.60	165.37±37.49
[redacted] w/v% the ingredient	2.28±1.12	116.97±26.41*
[redacted] w/v% the ingredient	2.43±1.06	78.26±34.52**

Mean±standard deviation (5 animals each)
†: p<0.01 (vs. vehicle control, Wilcoxon rank sum test)
††: p<0.01 (vs. vehicle control, t-test)
*: p<0.05 (vs. vehicle control, Dunnett's multiple comparison test)
**: p<0.01 (vs. vehicle control, Dunnett's multiple comparison test)

The results of a similar study in hairless mice (8 females per group) using the ingredient ([redacted] and [redacted] w/v%) solution, the vehicle control (ethanol), or positive control (0.1 w/v% retinol) are shown in Table 9.

Table 9 Wrinkle score and wrinkle length

Agent	Wrinkle score	Wrinkle length (mm/cm^2)
Vehicle control	4.20±0.32	35.44±3.38
0.1 w/v% retinol	2.52±0.37†	7.11±1.57††
[redacted] w/v% the ingredient	3.56±0.29	21.74±2.34**
[redacted] w/v% the ingredient	2.86±0.32*	16.08±2.64**

Mean±standard error (8 animals each)
†: p<0.05 (vs. vehicle control, Wilcoxon rank sum test)
*: p<0.05 (vs. vehicle control, Steel's multiple comparison test)
††: p<0.01 (vs. vehicle control, t-test)
**: p<0.01 (vs. vehicle control, Dunnett's multiple comparison test)

The above test results revealed that there was a trend of an improvement in the wrinkle score at the concentrations of [redacted] w/v% or more of the ingredient compared to the vehicle control group, and there was a significant difference in the wrinkle length from the vehicle control group, showing an improvement of wrinkles.

7) Improving effects on dermal collagen fiber bundles: Attachment E-1-4-(2)

Hairless mice (8 females per group) were irradiated with ultraviolet rays[15] [redacted] time(s) a week for [redacted] weeks, and 100 μL of the ingredient ([redacted] and [redacted] w/v%) solution or the vehicle control (ethanol) was administered percutaneously [redacted] time(s) a week for 8 weeks from the next day after completion of the irradiation period. After completion of the treatment, the collagen fiber bundles in the cross-section of the skin were photographed with a scanning electron microscope (SEM), and evaluation was performed using the score according to the collagen score criteria.[17] The results are shown in Table 10. A significant improvement of collagen bundle morphology was observed in the ingredient group

[17] Grade 0, complete bundles of collagen across the image; Grade 1, complete bundles of collagen over at least 50% of the image; Grade 2, complete bundles of collagen slightly found; Grade 3, no complete bundles of collagen

compared to the vehicle control group.

Table 10 Collagen fiber bundle score evaluation

Agent	Collagen fiber bundle score
Vehicle control	1.56 ± 0.20
[redacted] w/v% the ingredient	0.47 ± 0.19**
[redacted] w/v% the ingredient	0.81 ± 0.13*

Mean±standard error (8 animals each)
**, p<0.01; *, p<0.05
(vs. vehicle control, Steel's multiple comparison test)

(2) Clinical trials

1) Efficacy evaluation of the product in humans: Attachment E-2-(1)

To evaluate the efficacy of the product against human wrinkles, a placebo-controlled, randomized, double-blind, comparative study (left-right comparison study) was conducted at a single center in Japan in healthy Japanese women with wrinkles at the corners of the eyes corresponding mainly to Grade 3 (obvious shallow wrinkles) to 5 (slightly deep wrinkles) in accordance with the "Guideline for Evaluation of Anti-wrinkle Products"[18] by the Japanese Cosmetic Science Society.

As for dosage and administration, a rice grain-sized application of the designated preparation was to be made at the corners of the left and right eye each after washing the face and using a lotion twice a day (morning and evening) for 24 weeks. Of 52 participants enrolled into this study, 48 participants were included in the analysis set, excluding 4 participants who failed to visit on the scheduled assessment day.

The main results of wrinkle grade evaluation (visual evaluation, photographic evaluation) and replica analysis,[19] efficacy endpoints, were as shown in Table 11. Regarding the wrinkle grade (photographic evaluation), a statistically significant difference was observed in the change of wrinkle grade at Weeks 12 and 24 when comparing application sites of the product and those of the placebo. In the replica analysis, there was a statistically significant difference in the change in the maximum depth of the largest wrinkle at Week 24 when comparing these sites.

[18] Journal of Japanese Cosmetic Science Society, 30(4):316-332,2008
[19] The replicas of the corners of the eyes were collected, and 3-dimensional measurement was performed using a wrinkle measurement and analysis equipment PRIMOS® (GFM, software version 5.0.75.4).

Table 11 Analysis results of efficacy endpoints

Endpoints		The product application site	Placebo application site	p-value
Change in wrinkle grade (visual evaluation) (mean wrinkle grade)	Week 0	- (3.93 ± 0.12)	- (3.99 ± 0.12)	-
	Week 12	-0.07 ± 0.02 (3.86 ± 0.12)	-0.06 ± 0.02 (3.93 ± 0.12)	-
	Week 24	-0.17 ± 0.03 (3.76 ± 0.12)	-0.20 ± 0.03 (3.79 ± 0.12)	p=0.5762[a)]
Change in wrinkle grade (photographic evaluation) (mean wrinkle grade)	Week 0	- (4.27 ± 0.11)	- (4.19 ± 0.10)	-
	Week 12	-0.04 ± 0.02 (4.23 ± 0.11)	0.06 ± 0.03 (4.24 ± 0.11)	p<0.05[a)]
	Week 24	-0.15 ± 0.04 (4.11 ± 0.10)	0.09 ± 0.04 (4.28 ± 0.10)	p<0.001[a)]
Change in maximum depth of largest wrinkle (μm) (Mean maximum depth of largest wrinkle [μm])	Week 0	- (207.6 ± 8.5)	- (214.6 ± 9.1)	-
	Week 12	-9.0 ± 5.0 (198.6 ± 9.8)	1.0 ± 5.3 (215.6 ± 10.4)	p=0.2127[b)]
	Week 24	-20.9 ± 6.5 (186.8 ± 8.6)	2.6 ± 5.4 (217.2 ± 9.2)	p<0.001[b)]

Mean±standard error (n=48)
a) Wilcoxon signed rank sum test
b) Paired t-test

Regarding safety, no adverse events were reported in any of the participants throughout the study period.

2) Efficacy evaluation of the product in humans: Attachment E-2-(2)

To evaluate the efficacy of the product against human wrinkles, a placebo-controlled, randomized, double-blind, comparative study (left-right comparison study) was conducted at a single center in Japan in healthy Japanese women with wrinkles at the corners of the eyes corresponding mainly to Grade 3 (obvious shallow wrinkles) to 5 (slightly deep wrinkles) in accordance with the Guideline for Evaluation of Anti-wrinkle Products.

As for dosage and administration, a rice grain-sized application of the designated preparation was to be made at the corners of the left and right eye each after washing the face and using a lotion twice a day (morning and evening) for 12 weeks. Of 70 participants enrolled into this study, 68 participants were included in the analysis set, excluding 2 participants who failed to visit on the scheduled assessment day.

The main results of wrinkle grade evaluation (visual evaluation, photographic evaluation) and replica analysis,[19] efficacy endpoints, were as shown in Table 12. Regarding all endpoints, a statistically significant difference was observed in the change at Week 12 when comparing application sites of the product and those of the placebo.

Table 12 Analysis results of efficacy endpoints

Endpoints		The product application site	Placebo application site	p-value
Change in wrinkle grade (visual evaluation) (mean wrinkle grade)	Week 0	- (3.79 ± 0.05)	- (3.80 ± 0.05)	-
	Week 6	-0.05 ± 0.01 (3.74 ± 0.05)	-0.02 ± 0.01 (3.78 ± 0.06)	p=0.120[a)]
	Week 12	-0.10 ± 0.02 (3.69 ± 0.05)	-0.02 ± 0.02 (3.78 ± 0.06)	p<0.01[a)]
Change in wrinkle grade (photographic evaluation) (mean wrinkle grade)	Week 0	- (3.86 ± 0.05)	- (3.88 ± 0.05)	-
	Week 6	-0.04 ± 0.02 (3.82 ± 0.05)	-0.01 ± 0.01 (3.87 ± 0.05)	p=0.159[a)]
	Week 12	-0.10 ± 0.02 (3.76 ± 0.05)	-0.02 ± 0.01 (3.86 ± 0.05)	p<0.001[a)]
Change in maximum depth of largest wrinkle (μm) (Mean maximum depth of largest wrinkle [μm])	Week 0	- (211.3 ± 6.1)	- (213.8 ± 7.4)	-
	Week 6	-6.1 ± 2.9 (205.2 ± 6.4)	-0.2 ± 2.9 (213.6 ± 8.3)	p=0.071[b)]
	Week 12	-12.8 ± 2.6 (198.5 ± 6.2)	0.9 ± 2.4 (214.8 ± 8.1)	p<0.001[b)]

Mean±standard error (n=68)
a) Wilcoxon signed rank sum test
b) Paired t-test

Regarding safety, no adverse events were reported in any of the participants throughout the study period.

Outline of the review by PMDA

Based on the submitted data, PMDA discussed the efficacy of the product at the Expert Discussion, including the following points. The expert advisors supported the following conclusion by PMDA, and PMDA has concluded that there is no particular problem with the efficacy of the product. On the basis of the submitted human use results showing no adverse events in any participants, PMDA has concluded that there is no particular problem with the safety of the product in human use (see D. Safety, Outline of the review by PMDA).

(1) Efficacy and indications of the product

The basic studies supporting the indications confirmed the mechanism of action of the ingredient, and the study using a hairless mouse photoaged model confirmed an improvement of wrinkles and dermal collagen fiber bundles by the ingredient.

In order to evaluate the efficacy of the product against wrinkles, the applicant conducted a clinical trial in participants with wrinkles at the corners of the eyes by using visual or photographic evaluation of wrinkle grade and wrinkle analysis parameters using skin replica as endpoints in accordance with the Guideline for Evaluation of Anti-wrinkle Products.

In the clinical trial in which the product was used for 24 weeks (Attachment E-2-(1)), changes from baseline to Week 24 in the wrinkle grade (photographic evaluation) and the maximum depth of the largest wrinkle (replica analysis) of wrinkles at the corners of the eyes showed a significant improvement in the application sites of the product compared to those of the base. However, the expert advisors pointed out

a concern as to whether general users can actually feel an improvement in wrinkles due to the product because no significant improvement was observed in the replica analysis in the change in the maximum depth of the largest wrinkle at Week 12 and there was no clear difference in the results of the questionnaire survey[20] conducted in participants at Week 24 between the product and base (the proportion of participants who felt wrinkles "slightly improved" or better was 97.9% with the product and 95.8% with base).

In consideration of the above review, the applicant conducted an additional 12-week clinical trial (Attachment E-2-(2)). In this study, the product significantly improved wrinkles compared to the base, and participants were considered to feel the effect of the product as shown below.

- Regarding the changes in the wrinkle grade (visual evaluation and photographic evaluation) and the maximum depth of the largest wrinkle (replica analysis) of wrinkles at the corners of the eyes at Week 12, a significant improvement of wrinkles was observed with the product compared to the base.
- In the questionnaire survey[21] conducted at Week 12, the proportion of participants who felt wrinkles "slightly improved" or better was 85.2% with the product and 57.3% with base, showing a higher proportion of participants who felt an improvement in wrinkles with the product.

PMDA's view on the efficacy and indications of the product is as described below.

Based on the results of the clinical trial in which the product was used for 24 weeks (Attachment E-2-(1)), changes up to Week 24 in the wrinkle grade (photographic evaluation) and the maximum depth of the largest wrinkle (replica analysis) showed a significant improvement in the application sites of the product compared to those of the base, and there was considered to be no major problem in efficacy. Although, as in the opinion from the expert advisors, the improvement in wrinkles by the product was not clearly demonstrated at Week 12, the additional clinical trial in which the product was used for 12 weeks showed an improvement over time in all endpoints. The study also showed that there were significant differences in all endpoints as compared to the base and there were significantly more participants who felt an improvement in wrinkles with the product at Week 12. Therefore, the effects of the product were considered to be demonstrated. Since there has been no experience of administration for more than 24 weeks in human use studies, we consider it necessary to collect information from long-term users after marketing.

The applicant described the indications of the product, a medicated cosmetic cream, as "suppresses the degradation of elastin and collagen and prevents and improves wrinkles caused by ultraviolet rays." However, since quasi-drugs are used by general users, it should not contain the mechanism of action that may be difficult for general users to understand, and the indications should be simple and easy to understand. Therefore, PMDA considered it appropriate to describe the indications of the product as "improves wrinkles."

[20] Selected from "much improved," "improved," "slightly improved," "slightly worse," "worse," or "much worse."
[21] Selected from "feeling much improved," "feeling improved," "feeling slightly improved," "feeling no change," "feeling slightly worse," "feeling worse," or "feeling much worse."

3. Overall Evaluation

Based on the above review, PMDA has concluded that the product may be approved as a quasi-drug cream for the following indications, and dosage and administration. Since the ingredient is a new active ingredient to be contained in quasi-drugs, we consider it appropriate to add the following condition.

[Indications]	Improves wrinkles. Keeps the skin healthy. Protects the skin. Prevents dryness of the skin. Note) The underlined part indicates the indications related to the active ingredient of the product.
[Dosage and administration]	Take an appropriate amount and apply it to the skin.
[Approval Condition]	Post-marketing surveillance on safety should be conducted for at least 2 years after approval.