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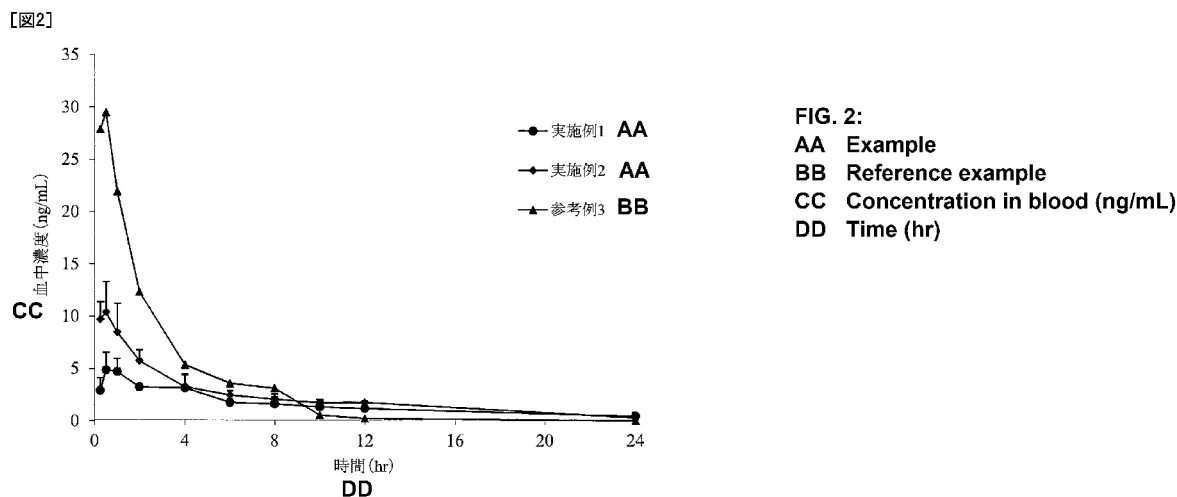


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(54) Title: POWDER PREPARATION FOR NASAL ADMINISTRATION

(54) 発明の名称: 粉末経鼻投与製剤



(57) Abstract: The purpose of the present invention is to prepare a powder preparation for nasal administration that includes, as an active ingredient, a particulate steroid hormone having an average particle size of 50 – 300 $\mu$ m. The powder preparation for nasal administration may further include a water-soluble polymer (in particular, a water-soluble polysaccharide such as cellulose comprising a hydroxyalkyl group). The particulate steroid hormone may be formed from testosterone and/or a derivative thereof. The water-soluble polymer may be particulate. The proportion of the water-soluble polymer may be around 1 – 50 parts by weight with respect to 1 part by weight of the particulate steroid hormone. This powder preparation for nasal administration may be configured such that



WO 2019/103108 A1

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the Cmax of the steroid hormone component can be adjusted to be 15ng/ml or less. The powder preparation for nasal administration enables the concentration of a steroid hormone such as testosterone in the blood to be constrained to a specific range over a long period of time.

(57) 要約: 有効成分として平均粒子径 50~300 $\mu$ m の粒子状ステロイドホルモン類を含む粉末経鼻投与製剤を調製する。前記粉末経鼻投与製剤は水溶性高分子(特にヒドロキシアルキル基を有するセルロースなどの水溶性多糖類)をさらに含んでもよい。前記粒子状ステロイドホルモン類はテストステロン及び/又はその誘導体で形成されていてもよい。前記水溶性高分子は粒子状であってもよい。前記水溶性高分子の割合は、前記粒子状ステロイドホルモン類1重量部に対して1~50重量部程度であってもよい。前記粉末経鼻投与製剤は、ステロイドホルモン類のC<sub>max</sub>を15ng/ml以下に調整できる粉末経鼻投与製剤であってもよい。テストステロンなどのステロイドホルモン類の血中濃度を特定の範囲に長期間に亘って制御できる粉末経鼻投与製剤を提供する。

DESCRIPTION

TITLE OF INVENTION

POWDER PREPARATION FOR NASAL ADMINISTRATION

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TECHNICAL FIELD

[0001] The present invention relates to a powder preparation for nasal administration containing steroid hormones such as testosterone effective in treating late-onset hypogonadism (LOH syndrome).

BACKGROUND ART

[0002] Late-onset hypogonadism (LOH syndrome) has various symptoms and signs such as cognitive impairment, sleep disorder, and increased visceral fat. LOH syndrome is associated with a decrease in blood testosterone concentration, and replacement of testosterone (17 $\beta$ -hydroxy-3-oxo-4-androstene) can be effective.

[0003] Conventional agents for testosterone replacement therapy are mainly injections and transdermal preparations. As problems with these preparations, injections involve pain of administration site and require hospital visits for administration, and implants also have the same disadvantages. Moreover, problems of transdermal preparations are contamination of family members who

have touched the applied site, dermatitis, and/or an increase in dihydrotestosterone (DHT). In either case, various side effects may occur due to a testosterone concentration rising to a non-physiological concentration immediately after administration.

[0004] Moreover, for nasal drops (liquid preparations) containing testosterone as an active ingredient, in order to take advantage of the fast-acting property that is a characteristic of transmucosal absorption, a method of reducing the particle size of testosterone to improve the in vivo absorption is attempted. Japanese Patent Application Laid-Open Publication No. H11-130659 (JP H11-130659 A, Patent Document 1) discloses an aqueous suspended pharmaceutical composition with an improved physical stability, containing testosterone as an effective amount of a drug and about 0.05 to 5% w/w crystalline cellulose and carboxymethylcellulose sodium (Avicel RC) as a suspending agent, wherein the drug particle has a spherical form with a particle size of 10 nm to 10  $\mu\text{m}$ . In Examples, testosterone of 0.1 to 3  $\mu\text{m}$  is used.

[0005] Although the liquid preparation containing testosterone with such a small particle size improves the absorption, the maximum plasma concentration ( $C_{\text{max}}$ ) of testosterone as a criterion of testosterone

replacement therapy by U.S. Food and Drug Administration (FDA) is over 15 ng/ml. For example, the same criterion is also set for the pharmaceutical "AVEED" used for testosterone replacement therapy in the U.S. Further, the average plasma concentration (C<sub>avg</sub>) of testosterone exceeds 2.01 to 7.5 ng/ml considered to be a plasma testosterone level (normal range) in adult male (Japanese Journal of Urology 95: 751-760, 2004), and there is concern about the occurrence of unexpected side effects. In particular, a rapid increase in blood testosterone concentration causes a subsequent rapid decrease in blood testosterone concentration, and is considered to also cause side effects such as polycythemia, elevated Prostate Specific Antigen (PSA), urinary retention, gynecomastia, and progression of sleep apnea.

[0006] Japanese Patent Application Laid-Open Publication No. 2012-526726 (JP 2012-526726 A, Patent Document 2) discloses an inhalable dry powder pharmaceutical preparation containing a therapeutic agent, wherein at least part of the therapeutic agent is present in a freebase form, and the therapeutic agent contains particles with a particle size distribution of about 5 to 250  $\mu\text{m}$ . This document describes, as a problem to be solved by invention, the necessity to be able to engineer inhalable pharmaceutical preparations so that the

pharmacokinetic profiles more closely fit clinical requirements. Specifically, this document describes, for patients with migraine, fast initial mitigation of the symptom by fast arrival of effective blood concentration or recurrence prevention by maintaining an effective blood concentration with time. In Examples, as an intranasal dry powder pharmaceutical preparation, a dry powder pharmaceutical preparation of sumatriptan or zolmitriptan that is a therapeutic agent for migraine is prepared (a free base single product with a particle size of 15  $\mu\text{m}$ , a mixture product of a free base with a particle size of 15  $\mu\text{m}$  and a free base having a particle size of 38 to 100  $\mu\text{m}$ ). Moreover, in Examples, although a granisetron dry powder pharmaceutical preparation is evaluated for the taste, the particle size of granisetron is not disclosed. Further, this document discloses that the dry powder pharmaceutical preparation may have no carrier or may be a mixture of a carrier and optional one or more excipients and the therapeutic agent. This document exemplifies many carriers and excipients, however, in Examples, this document does not mention a carrier and an excipient.

[0007] Japanese Patent Application Laid-Open Publication No. H09-291025 (JP H09-291025 A, Patent Document 3) discloses a powder composition for nasal administration comprising a drug, an absorbent and

gel-forming vehicle such as a hydroxypropylcellulose,  
and an absorbent and poorly water-soluble vehicle  
such as a crystalline cellulose. This document aims  
to improve an absorption of a drug and to increase a  
5 maximum blood concentration of a water-soluble drug,  
a drug other than a hydrophobic drug, or a nonpeptide  
and/or nonprotein drug. Regarding the particle size  
of the drug, although this document discloses that it  
is preferred that not less than 90% by weight of the  
10 particle have a particle size of 10 to 350  $\mu\text{m}$ , the  
particle size in Examples is unknown. In Examples, as  
the above-mentioned drug, beclometasone dipropionate  
being an anti-inflammatory steroid, metoclopramide  
being an antiemetic, leuprolide acetate being a  
15 luteinizing peptide hormone, and salmon calcitonin  
being a peptide hormone are used.

[0008] As described above, the conventional  
technology currently examines the development of the  
agents for testosterone replacement therapy from  
20 various viewpoints on the assumption that the  
particle size of testosterone is reduced as found in  
Patent Document 1. There is no attempt to control the  
blood testosterone concentration in the normal range  
by adjusting the particle size of testosterone (in  
25 particular, for a nasal powder preparation).

#### CITATION LIST



PATENT LITERATURE

[0009] Patent Document 1: JP H11-130659 A (claims 1 and 6, and Example 3)

Patent Document 2: JP 2012-526726 A (claim 1, paragraphs [0002], [0003], [0131] to [0134], [0156], and [0178], and Examples)

Patent Document 3: JP H09-291025 A (claim 1, paragraphs [0019], [0022], and [0048], and Examples)

10

SUMMARY OF INVENTION

[0010] It would be advantageous to provide a powder preparation for nasal administration which can control a plasma concentration of steroid hormones such as testosterone in a specific range over a long period of time.

[0011] It would also be advantageous to provide a powder preparation for nasal administration which inhibits a rapid increase in plasma concentration and reduces a frequency of administration and side effects.

[0012] It would also be advantageous to provide a powder preparation for nasal administration which can control a maximum plasma concentration ( $C_{max}$ ) of testosterone to a plasma testosterone level for adult male and can adjust or control an average plasma testosterone concentration ( $C_{avg}$ ) to a plasma testosterone level for adult male.

[0013] The inventors of the present invention made intensive studies and found that a plasma concentration of steroid hormones such as testosterone can be controlled in a specific range over a long period of time by adjusting or  
5 controlling an average particle size of a particulate of steroid hormones (or particulate steroid hormones) to 50 to 300  $\mu\text{m}$ , the steroid hormones being an active ingredient of a powder preparation for nasal  
10 administration. The present invention was accomplished based on the above findings.

[0014] That is, the present invention provides a powder (or powdery) preparation for nasal administration containing a particulate of steroid  
15 hormones (or particulate steroid hormones, particle of steroid hormones, steroid hormone particle) having an average particle size of 50 to 300  $\mu\text{m}$  as an active ingredient. The powder preparation for nasal administration may further contain a water-soluble  
20 polymer (in particular, water-soluble polysaccharides such as a cellulose having a hydroxyalkyl group). The water-soluble polymer may be free from a crystalline cellulose or a carboxymethylcellulose sodium. The water-soluble polymer may be in a particulate form.  
25 The steroid hormones may contain (or may be formed of) a male hormone (in particular, testosterone and/or a derivative thereof). The ratio of the water-

soluble polymer may be about 1 to 50 parts by weight relative to 1 part by weight of the particulate of steroid hormones. The powder preparation for nasal administration may be a powder preparation for nasal administration which can control (or maintain or adjust) a maximum plasma concentration ( $C_{max}$ ) of the steroid hormones to 15 ng/ml or less. The powder preparation for nasal administration may be a powder preparation for nasal administration which can control (or maintain or adjust) an average plasma concentration ( $C_{avg}$ ) of the steroid hormones to 2 to 7.5 ng/ml.

[0014a] The present invention as claimed herein is described in the following Items 1 to 10:

[Item 1] A powder preparation for nasal administration, containing steroid hormones being in a particulate form and having an average particle size of 50 to 300  $\mu\text{m}$  as an active ingredient, wherein the powder preparation controls an average plasma concentration ( $C_{avg}$ ) of the steroid hormones to 2 to 7.5 ng/ml.

[Item 2] The powder preparation for nasal administration according to item 1, which further contains a water-soluble polymer.

[Item 3] The powder preparation for nasal administration according to item 2, wherein the water-soluble polymer contains water-soluble polysaccharides.

[Item 4] The powder preparation for nasal administration according to item 3, wherein the water-soluble polysaccharides contain a cellulose having a hydroxyalkyl group.

[Item 5] The powder preparation for nasal administration according to any one of items 2 to 4, wherein the water-soluble polymer is free from a crystalline cellulose and a carboxymethylcellulose sodium.

[Item 6] The powder preparation for nasal administration according to any one of items 2 to 5, wherein the water-soluble polymer is in a particulate form.

[Item 7] The powder preparation for nasal administration according to any one of items 1 to 6, wherein the steroid hormones are a male hormone.

[Item 8] The powder preparation for nasal administration according to item 7, wherein the male

hormone is at least one selected from testosterone, a C<sub>1-4</sub>alkyltestosterone, a C<sub>1-18</sub>alkanoic acid ester of testosterone, a cycloalkanoic acid ester of testosterone, dihydrotestosterone and

5 dehydroepiandrosterone.

[Item 9] The powder preparation for nasal administration according to any one of items 2 to 8, wherein a ratio of the water-soluble polymer is 1 to 50 parts by weight relative to 1 part by weight of

10 the steroid hormones.

[Item 10] The powder preparation for nasal administration according to any one of items 1 to 9, which controls a maximum plasma concentration (C<sub>max</sub>) of the steroid hormones to not more than 15 ng/ml.

15

#### ADVANTAGEOUS EFFECTS OF INVENTION

[0015] According to the present invention, since the average particle size of the particulate of steroid hormones (or particulate steroid hormones) being an

20 active ingredient of the powder preparation for nasal administration is adjusted to 50 to 300  $\mu\text{m}$ , the plasma concentration of the steroid hormones such as testosterone can be controlled in the specific range over a long period of time. This allows inhibition of

25 a rapid increase in blood concentration and reduction

of a frequency of administration and side effects.  
For example, the side effects can also be reduced by  
administration twice per day. In particular, a  
combination testosterone as the steroid hormones and  
5 the specific water-soluble polymer can control the  
maximum plasma concentration ( $C_{max}$ ) of testosterone to  
15 ng/ml or less and can control the average plasma  
concentration ( $C_{avg}$ ) of the steroid hormones to 2 to  
7.5 ng/ml.

10

#### BRIEF DESCRIPTION OF DRAWINGS

[0016] [Fig. 1] Fig. 1 is a graph showing a change  
over time of a plasma testosterone concentration in  
each powder preparation for nasal administration  
15 obtained in Reference Examples 1 and 2.

[Fig. 2] Fig. 2 is a graph showing a change  
over time of a plasma testosterone concentration in  
each powder preparation for nasal administration  
obtained in Examples 1 to 2 and Reference Example 3.

20 [Fig. 3] Fig. 3 is a graph showing a change  
over time of a plasma testosterone concentration in  
each powder preparation for nasal administration  
obtained in Examples 3 to 5 and Reference Example 4.

[Fig. 4] Fig. 4 is a graph showing a change  
25 over time of a plasma testosterone concentration in  
each powder preparation for nasal administration  
obtained in Examples 6 to 8.

[Fig. 5] Fig. 5 is a graph showing a change over time of a plasma testosterone concentration in each powder preparation for nasal administration obtained in Examples 9 to 12.

5 [Fig. 6] Fig. 6 is a graph showing a change over time of a plasma testosterone concentration in each powder preparation for nasal administration obtained in Examples 13 to 15.

[Fig. 7] Fig. 7 is a graph showing a change  
10 over time of a plasma testosterone concentration in each powder preparation for nasal administration obtained in Examples 16 to 21.

[Fig. 8] Fig. 8 is a graph showing a change  
15 over time of a plasma testosterone concentration in each powder preparation for nasal administration obtained in Examples 22 to 27.

#### DESCRIPTION OF EMBODIMENTS

[0017] [Active ingredient]

20 The powder (or powdery) preparation for nasal administration of the present invention contains a particulate of steroid hormones (particulate steroid hormones, steroid hormone particulates, steroid hormone particles) having an average particle size of  
25 50 to 300  $\mu\text{m}$  as an active ingredient (a pharmacologically or biologically active ingredient). The steroid hormones are classified roughly into male

hormones (androgen), female hormones (estrogen), and corticoids. The male hormone may include, for example, testosterone, a testosterone derivative [for example, a C<sub>1-4</sub>alkyltestosterone such as

5 methyltestosterone; a C<sub>1-18</sub>alkanoic acid ester of testosterone such as testosterone acetate, testosterone propionate, testosterone isocaproate, testosterone enanthate, testosterone decanoate, or testosterone undecanoate; a cycloalkanoic acid ester

10 of testosterone such as testosterone butylhexanoate (Testosterone buciclate); dihydrotestosterone (DHT); and dehydroepiandrosterone (DHEA)], and drostanolone. Examples of the female hormones may include an estrogen such as estradiol, estriol, estrone, or

15 fosfestrol, and a gestagen such as progesterone, norethisterone, or pregnanediol. The corticoids may include, for example, dexamethasone phosphate, fludrocortisone acetate, methylprednisolone sodium succinate, and hydrocortisone phosphate. These

20 steroid hormones may be used alone or in combination. [0018] Among them, a steroid hormone (in particular, a male hormone) is preferred, and testosterone or a derivative thereof (in particular, testosterone) is particularly preferred.

25 [0019] The form (or shape) of the particulate of steroid hormones is any particulate (or powdery) form without limitation to a specific one, and may be an



isotropic form (such as a spherical or substantially spherical form or a cubic form) or may be an anisotropic form (such as an ellipsoidal form, a polyhedral form, a rectangular parallelepiped form, a fiber form, or an amorphous form).

[0020] According to the present invention, since the particulate of steroid hormones has a specific average particle size and has a particle size adjusted in a specific range, the plasma concentration of the steroid hormones can be controlled in a specific range for a long period of time. The volume average particle size (D50) of the steroid hormones is 50 to 300  $\mu\text{m}$  and may be preferably about 70 to 250  $\mu\text{m}$  and more preferably about 100 to 230  $\mu\text{m}$  (for example, about 150 to 200  $\mu\text{m}$ ). From the viewpoint of inhibiting a rapid increase in plasma concentration and reducing a frequency of administration and side effects, the volume average particle size may be, for example, about 50 to 200  $\mu\text{m}$ , preferably about 80 to 150  $\mu\text{m}$ , and more preferably about 90 to 130  $\mu\text{m}$  (particularly about 100 to 120  $\mu\text{m}$ ). In a case where the average particle size is excessively large, the powder preparation for nasal administration provides a low maximum plasma concentration ( $C_{\text{max}}$ ) of the steroid hormones. In a case where the average particle size is excessively small, the powder preparation for

nasal administration provides a low average plasma concentration ( $C_{avg}$ ) of the steroid hormones. Herein and in the claims, in a case where the form of the particulate of steroid hormones is an anisotropic form, the particle size of each particle means an average value of a major axis and a minor axis.

[0021] It is preferred that the steroid hormones preferably have a narrower particle size distribution (particle size distribution). The particle size ( $D_{90}$ ) at a cumulative frequency of 90% is about 100 to 300  $\mu\text{m}$ , preferably about 120 to 280  $\mu\text{m}$ , and more preferably about 150 to 250  $\mu\text{m}$  (particularly about 200 to 240  $\mu\text{m}$ ).

[0022] A method of preparing steroid hormones having a narrow particle size distribution (a method of controlling a particle size) may use a conventional powder processing apparatus. For example, uniform particles may be prepared using a commercially available surface modification processing apparatus ("NOBILTA NOB-MINI" manufactured by HOSOKAWA MICRON CORPORATION).

[0023] Herein and in the claims, the volume average particle size and the particle size distribution of the particulate of steroid hormones can be measured using a laser diffraction particle size analyzer, specifically, can be measured according to the method described in Examples mentioned below.

[0024] The active ingredient may contain other active ingredients in addition to the particulate of steroid hormones. Other active ingredients are any active ingredient having a pharmacological action and may include, but should not be limited to, for example, other hormones such as pancreatic circulatory hormones, prostaglandins, steroids, corticoids, thyroid hormones, and growth hormones.

[0025] The proportion of the particulate of steroid hormones in the active ingredient may be, for example, not less than 50% by weight, may be preferably not less than 80% by weight, more preferably not less than 90% by weight, or may be 100% by weight (the active ingredient contains only the particulate of steroid hormones).

[0026] [Water-soluble polymer]

The powder preparation for nasal administration of the present invention further contains a water-soluble polymer in addition to the active ingredient. According to the present invention, a combination of the active ingredient having the above-mentioned particle size and the water-soluble polymer allows further effective control (or adjustment) of the plasma concentration of the steroid hormones.

[0027] The water-soluble polymer may include a water-soluble synthetic polymer, water-soluble

polysaccharides, or others. The water-soluble synthetic polymer may be used alone, the water-soluble polysaccharides may be used alone, or the water-soluble synthetic polymer and the water-soluble polysaccharides may be used in combination.

[0028] The water-soluble synthetic polymer may include, for example, a copolyvidone such as a polyvinylpyrrolidone (PVP) or Kollidon (a copolymer of vinylpyrrolidone and vinyl acetate), a polyvinyl alcohol (PVA), a carboxyvinyl polymer, a polyacrylic polymer, and a polyethylene glycol. These water-soluble synthetic polymers may be used alone or in combination.

[0029] The water-soluble polysaccharides are pharmacologically or physiologically acceptable polysaccharides. Examples of the polysaccharides may include a soluble starch such as a pregelatinized starch, a partially pregelatinized starch, or a sodium carboxymethylstarch; cellulose ethers such as a methylcellulose (MC), a carboxymethylcellulose (carmellose or CMC), a carboxymethylcellulose sodium (CMC-Na), a carboxymethylcellulose calcium, a hydroxyethylcellulose (HEC), a hydroxypropylcellulose (HPC), and a hydroxypropylmethylcellulose (HPMC); cellulose esters such as a cellulose phthalate and a hydroxypropylmethylcellulose phthalate; homopolysaccharides such as a chitin, a chitosan, and

a pullulan; and heteropolysaccharides such as a gum arabic (gum acacia), a xanthan gum, a locust bean gum, a tragacanth gum, and a sodium alginate. These water-soluble polysaccharides may be used alone or in  
5 combination.

[0030] Among these water-soluble polymers, a water-soluble polymer which forms a gel with water and easily adheres to the nasal mucosa is preferred. For example, the above-exemplified water-soluble  
10 synthetic polymer and water-soluble polysaccharides are preferred. Specifically, the water-soluble polysaccharides (such as MC, CMC, CMC-Na, HEC, HPC, and HPMC) are preferred, and a cellulose having a hydroxyalkyl group (in particular, hydroxypropyl  
15 group) (e.g., a hydroxyC<sub>2-4</sub>alkylcellulose such as HEC or HPC, and a hydroxyC<sub>2-4</sub>alkylC<sub>1-4</sub>alkylcellulose such as HPMC) is particularly preferred. Among them, a combination of a hydroxyalkylcellulose (in particular, a hydroxyC<sub>2-3</sub>alkylcellulose such as a  
20 hydroxypropylcellulose) and the particulate of steroid hormones having the above-mentioned particle size (in particular, testosterone) allows effective control (or adjustment) of the average plasma concentration (C<sub>avg</sub>), and thus inhibition of an  
25 excessive increase in the maximum plasma concentration (C<sub>max</sub>) and control (or adjustment) of the average plasma concentration (C<sub>avg</sub>) are

compatible.

[0031] The proportion of the cellulose having a hydroxyalkyl group (for example, a cellulose having hydroxypropyl group, such as a hydroxypropylcellulose or a hydroxypropylmethylcellulose) in the water-soluble polysaccharides may be, for example, not less than 50% by weight, may be preferably not less than 80% by weight and more preferably not less than 90% by weight, or may be 100% by weight (the water-soluble polysaccharides contain only the cellulose having a hydroxyalkyl group). According to the present invention, the polysaccharides may be free from a crystalline cellulose.

[0032] The water-soluble polymer (in particular, the water-soluble polysaccharides such as a hydroxypropylcellulose), e.g., for 2% by weight aqueous solution at 20°C may have a viscosity of, for example, about 2 to 10000 mPa·s, preferably about 10 to 8000 mPa·s (e.g., about 100 to 7000 mPa·s), and more preferably about 120 to 5000 mPa·s (particularly about 150 to 4000 mPa·s). The above-mentioned viscosity may be selected depending on the application, for example, may be about 50 to 1000 mPa·s (particularly about 100 to 500 mPa·s). In a case where the handleability is concerned, for example, the viscosity may be about 500 to 5000 mPa·s (particularly about 1000 to 4000 mPa·s). A water-

soluble polymer having an excessive low viscosity may have a low handleability. Herein and in the claims, the viscosity can be measured by a capillary viscometer method or a rotational viscometer method.

5 [0033] The number-average molecular weight (Mn) of the water-soluble polymer (in particular, the water-soluble polysaccharides such as a hydroxypropylcellulose) may be, for example, about 100,000 to 5,000,000, preferably about 300,000 to 10 2,000,000, and more preferably about 500,000 to 1,500,000 in terms of a polystyrene using GPC (gel permeation chromatography). The molecular weight may be selected depending on the application, for example, may be about 500,000 to 1,000,000 15 (particularly about 600,000 to 800,000). In a case where the handleability is concerned, for example, the molecular weight may be about 700,000 to 1,500,000 (particularly about 800,000 to 1,000,000).

[0034] The form (or shape) of the water-soluble 20 polymer is not particularly limited to a specific one. For example, the form of the water-soluble polymer may be a particulate form that is independent of the particulate of steroid hormones, may be a composite form with the steroid hormones (e.g., a 25 form covering or adhering to the surface of the steroid hormones), or may be a combination of the particulate form and the composite form. Among them,

the particulate form is preferred.

[0035] The form (or shape) of the particulate water-soluble polymer (or the particulate of water-soluble polymer) is not particularly limited to a specific one and may be an isotropic form (such as a spherical or substantially spherical form, or a cubic form) or may be an anisotropic form (such as an ellipsoidal form, a polyhedral form, a rectangular parallelepiped form, a fiber form, or an amorphous form). Among them, the isotropic form or a form near the isotropic form is preferred. The ratio of the major axis relative to the minor axis may be, for example, not more than 100 or may be preferably not more than 10 and more preferably not more than 5 (particularly not more than 3).

[0036] The particulate water-soluble polymer (in particular, the water-soluble polysaccharides such as a hydroxypropylcellulose) may have a volume average particle size (D50) of, for example, about 10 to 500  $\mu\text{m}$ , preferably about 30 to 300  $\mu\text{m}$ , and more preferably about 40 to 200  $\mu\text{m}$  (particularly about 50 to 150  $\mu\text{m}$ ). Moreover, the average particle size of the particulate water-soluble polymer may be, for example, about 0.1 to 10 times, preferably about 0.2 to 5 times (e.g., about 0.3 to 1 time), and more preferably about 0.4 to 0.8 times (particularly about 0.5 to 0.7 times) as large as the average particle



size of the particulate of steroid hormones. A particulate water-soluble polymer having an excessively large average particle size may have difficulty in uniformly mixing with the particulate of hormones. A particulate water-soluble polymer having an excessively small average particle size may also have difficulty in uniformly mixing with the particulate of hormones. The volume average particle size of the particulate water-soluble polymer can be measured by the same method as that for the average particle size of the particulate of steroid hormones.

[0037] The ratio of the water-soluble polymer relative to 1 part by weight of the particulate of steroid hormones is, for example, about 1 to 50 parts by weight, preferably about 3 to 30 parts by weight, and more preferably about 4 to 20 parts by weight (particularly about 4 to 15 parts by weight). In a case where the ratio of the water-soluble polymer is excessively low, it may be impossible to effectively control (or adjust) the average plasma concentration ( $C_{avg}$ ).

[0038] [Other ingredients]

The powder preparation for nasal administration of the present invention may further contain an excipient in addition to the active ingredient and the water-soluble polymer. The excipient may include, for example, saccharides or

sugar alcohols such as lactose, white sugar or refined sugar, glucose, sucrose, mannitol, sorbitol, and xylitol; and a microcrystalline cellulose. These excipients may be used alone or in combination. The  
5 ratio of the excipient relative to 100 parts by weight of the particulate of steroid hormones is, for example, about 0.1 to 100 parts by weight, preferably about 1 to 50 parts by weight, and more preferably about 3 to 30 parts by weight.

10 [0039] The powder preparation for nasal administration of the present invention may further contain other ingredients if necessary. Other ingredients are pharmacologically or physiologically acceptable ingredients. Examples of other ingredients  
15 may include a binder (for example, starches such as a corn starch and a dextrin; celluloses such as a crystalline cellulose (also including a microcrystalline cellulose) and an ethylcellulose (EC); and a synthetic polymer such as a polylactic  
20 acid), a disintegrant (for example, calcium carbonate, a croscarmellose sodium, and a crosslinked polyvinylpyrrolidone), a lubricant, a disintegrant aid, an antioxidant or an oxidation inhibitor, a stabilizer, an antiseptic agent or a preservative, a  
25 fungicide or an antibacterial agent, an antistatic agent, a corrigent or a masking agent, a coloring agent, a deodorant or a perfume, an algefacient, and

an antifoaming agent. These other ingredients may be used alone or in combination. The ratio of other ingredients relative to 100 parts by weight of the particulate of steroid hormones is, for example,  
5 about 0.1 to 100 parts by weight, preferably about 1 to 50 parts by weight, and more preferably about 3 to 30 parts by weight.

[0040] [Characteristics of powder preparation for nasal administration]

10           The powder preparation for nasal administration of the present invention is in a powder (particulate) form containing the above-mentioned particulate of steroid hormones. In a case where the water-soluble polymer is combined with the  
15 particulate of steroid hormones, the preparation may be a composite particle in which the water-soluble polymer is compound with the particulate of steroid hormones, may be a mixture of the particulate of steroid hormones and the particulate water-soluble  
20 polymer, or may be a mixture of the composite particle, the particulate of steroid hormones, and the particulate water-soluble polymer.

[0041] As a method of producing the composite particle, there may be used a conventional  
25 granulation method. The granulation method may be either a dry granulation method (for example, a dry shredding granulation method and a compression

molding granulation method) or a wet granulation method (for example, an extruding granulation method, a tumbling granulation method, a fluidized bed granulation method, a mixing agitation granulation method, a spray drying granulation method, and a vibration granulation method). Among them, the wet granulation method or other methods are widely used. The obtained granule may be pulverized and/or sized if necessary. The composite particle may have a volume average particle size (D50) of, for example, about 100 to 500  $\mu\text{m}$ , preferably about 120 to 300  $\mu\text{m}$ , and more preferably about 150 to 250  $\mu\text{m}$ . The volume average particle size of the composite particle can be measured by the same method as that for the average particle size of the particulate of steroid hormones.

[0042] The method of producing the particulate of steroid hormones and that of producing the particulate water-soluble polymer may be any of the above-mentioned dry granulation method and the above-mentioned wet granulation method. Among the granulation methods, the dry shredding granulation method is widely used in terms of simplicity or others. For the pulverization, there may be usually employed a pulverizer used for pulverization of pharmaceuticals, for example, an atomizer (such as a sample mill or a hammer mill), a pin mill, a jet

mill, and a ball mill.

[0043] As the method of mixing the mixture, there may be used a conventional method, for example, a method using a rotary vessel mixer such as a V-shaped mixer or a container mixer, a fixed vessel mixer such as a ribbon mixer or a high-speed mixer, or other mixers. The powder preparation for nasal administration of the present invention is preferably a mixture of the particulate of steroid hormones and the particulate water-soluble polymer. In this mixture, it is preferred that the particulate of steroid hormones and the particulate water-soluble polymer be mixed uniformly.

[0044] The powder preparation for nasal administration of the present invention can inhibit an excessive increase in the maximum plasma concentration ( $C_{max}$ ) of the steroid hormones (in particular, testosterone). The maximum plasma concentration ( $C_{max}$ ) may be, for example, not more than 25 ng/ml, preferably not more than 18 ng/ml, and more preferably not more than 15 ng/ml (particularly not more than 7.5 ng/ml).

[0045] The powder preparation for nasal administration of the present invention can also control (or reduce) the change (the average plasma concentration ( $C_{avg}$ )) of the plasma concentration of the steroid hormones (in particular, testosterone).

For example, the powder preparation can maintain the average plasma concentration ( $C_{avg}$ ) within the range from 1 to 10 ng/ml, preferably 2 to 7.5 ng/ml, and more preferably 2.5 to 7 ng/ml. Further, the control  
5 of the change can be maintained over 12 hours.

[0046] Since the powder preparation for nasal administration of the present invention has a small change of the blood concentration of the steroid hormones, the steroid hormones (in particular,  
10 testosterone) have a late time ( $T_{max}$ ) to reach the maximum plasma concentration ( $C_{max}$ ). For example, the  $T_{max}$  may be not less than 0.1 hours (e.g., 0.1 to 3 hours), preferably not less than 0.3 hours, and more preferably not less than 0.5 hours (particularly not  
15 less than 0.8 hours).

[0047] Since the powder preparation for nasal administration of the present invention of the present invention has a small change of the plasma concentration of the steroid hormones, the steroid  
20 hormones (in particular, testosterone) also have a long elimination half-life ( $t_{1/2}$ ), where the elimination half-life ( $t_{1/2}$ ) is the time required for the plasma concentration to reach half of the maximum plasma concentration ( $C_{max}$ ). For example, the  $t_{1/2}$   
25 may be not less than 2 hours (e.g., 2 to 10 hours), preferably not less than 3 hours, and more preferably not less than 5 hours (particularly not less than 6

hours).

[0048] The powder preparation for nasal administration of the present invention also allows the control (or adjustment) of the average plasma concentration ( $C_{avg}$ ) of the steroid hormones (in particular, testosterone). For example, the powder preparation can control (or adjust) the average plasma concentration ( $C_{avg}$ ) to 3 to 10 ng/ml that is the endpoint of testosterone replacement therapy clinical trial shown by FDA.

[0049] The powder preparation for nasal administration of the present invention has a long mean residence time in plasma ( $MRT_{0-\infty}$ ). For example, the mean residence time in plasma ( $MRT_{0-\infty}$ ) may be not less than 3 hours (e.g., about 3 to 20 hours) or may be, for example, not less than 4 hours, preferably not less than 5 hours, and more preferably not less than 6 hours (particularly not less than 7 hours).

[0050] Herein and in the claims, the maximum plasma concentration ( $C_{max}$ ), the time to reach the maximum plasma concentration ( $T_{max}$ ), the elimination half-life ( $t_{1/2}$ ), the average plasma concentration ( $C_{avg}$ ), and the mean residence time in plasma ( $MRT_{0-\infty}$ ) can be evaluated according to the methods described in the after-mentioned Examples.

[0051] Since the powder preparation for nasal administration of the present invention inhibits an

excessive increase in the maximum plasma concentration ( $C_{max}$ ) and has less side effects, the powder preparation also has an excellent safety.

Thus, the powder preparation can safely be administered to human beings and non-human animals, usually, mammals (for example, human beings, rabbits, dogs, cats, bovines, horses, pigs, and monkeys).

[0052] The powder preparation for nasal administration of the present invention is in any powdery form that may be powders, fine granules, granules, sprays, and aerosols. The method of administration is any administration to the nasal cavities such as spraying and may be selected depending to the species of the preparation without specific limitation.

[0053] The frequency of administration is not particularly limited to a specific one. For example, the frequency of administration may be once per day or may be a plurality of times per day (e.g., twice to three times per day) if necessary. Since the excessive increase in the maximum plasma concentration ( $C_{max}$ ) is inhibited and the plasma testosterone concentration is stabilized over 12 hours, the frequency of administration may be twice per day.

[0054] The amount to be administered (or dose) may be selected according to the species, age, body weight,



and condition (e.g., a performance status, a  
condition of a disease, a presence of a complication)  
of the subject to be administered, the duration (or  
period or schedule) of administration, the dosage  
5 form, the method (or route) of administration, and  
others. For example, the amount to be administered  
(or dose) to human beings (daily dose) is about 0.01  
to 50 mg/day, preferably about 0.05 to 30 mg/day  
(e.g., about 0.1 to 20 mg/day), more preferably about  
10 0.5 to 15 mg/day (particularly about 1 to 10 mg/day).

#### EXAMPLES

[0055] The following examples are intended to  
describe this invention in further detail and should  
15 by no means be interpreted as defining the scope of  
the invention. Incidentally, the details of raw  
materials used and the method of measuring an average  
particle size are as follows.

[0056] [Raw materials]

20 Testosterone A: testosterone manufactured by  
Tokyo Chemical Industry Co., Ltd., volume average  
particle size (D50) 123  $\mu\text{m}$

Testosterone B: testosterone manufactured by  
SIGMA-ALDRICH, volume average particle size (D50) 184  
25  $\mu\text{m}$

Testosterone C: testosterone manufactured by  
Bayer, volume average particle size (D50) 273  $\mu\text{m}$

[0057] Hydroxypropylcellulose H: "HPC-H" manufactured by Nippon Soda Co., Ltd., volume average particle size (D50) 80 to 110  $\mu\text{m}$ , viscosity (2% by weight aqueous solution, 20°C) 1000 to 4000 mPa·s, molecular weight (GPC method) 910,000

Hydroxypropylcellulose M: "HPC-M" manufactured by Nippon Soda Co., Ltd., viscosity (2% by weight aqueous solution, 20°C) 150 to 400 mPa·s, molecular weight (GPC method) 700,000

10 Hydroxypropylcellulose L: "HPC-L" manufactured by Nippon Soda Co., Ltd., viscosity (2% by weight aqueous solution, 20°C) 6 to 10 mPa·s, molecular weight (GPC method) 140,000

[0058] Crystalline cellulose: "CEOLUS PH-F20JP" manufactured by Asahi Kasei Chemicals Corp.

[0059] Pregelatinized starch: "MX-1" manufactured by Asahi Kasei Chemicals Corp., viscosity (2% by weight aqueous solution, 25°C) 70 mPa·s

20 Carboxyvinyl polymer: "974P" manufactured by Lubrizol Corporation, viscosity (2% by weight, pH 7.5) 294,000 to 394,000 mPa·s, molecular weight 500,000 to 5,000,000

Sodium alginate: "I-8" manufactured by KIMICA Corporation, viscosity (1% by weight aqueous solution, 20°C) 800 to 900 mPa·s, molecular weight 1,000,000 to 4,000,000

Hydroxypropylmethylcellulose: "90SH-15000SR"

manufactured by Shin-Etsu Chemical Co., Ltd.,  
viscosity (2% by weight aqueous solution, 20°C) 15,000  
mPa·s, molecular weight 10,000 to 200,000

Polyvinylpyrrolidone: "K-90" manufactured by  
5 BASF, viscosity (10 w/v% aqueous solution, 20°C) 300  
to 700 mPa·s, molecular weight 360,000

Xanthan gum: "CG-F" manufactured by Sansho  
Co., Ltd., viscosity (1% by weight aqueous solution,  
addition of 1% by weight KCl) 1200 to 1600 mPa·s,  
10 molecular weight 2,000,000 to 50,000,000

[0060] Polyethylene glycol: "6000" manufactured by  
Sanyo Chemical Industries, Ltd., viscosity (500 g/L  
aqueous solution, 25°C) 200 to 400 mPa·s, molecular  
weight 7300 to 9300

15 Polyvinyl alcohol: "EG-18" manufactured by The  
Nippon Synthetic Chemical Industry Co., Ltd.,  
viscosity (4% by weight aqueous solution) 18.3 mPa·s,  
molecular weight 30,000 to 110,000

Chitosan: "F" manufactured by KIMICA  
20 Corporation, viscosity (0.5% by weight aqueous  
solution, 20°C) 5 to 20 mPa·s, molecular weight 10,000  
to 1,000,000

Carmellose sodium: "PR-S" manufactured by DKS  
Co. Ltd., viscosity (2% by weight aqueous solution,  
25 25°C) 20 to 40 mPa·s, molecular weight 6,000 to 30,000

Locust bean gum: "RL-200Z" manufactured by  
KIMICA Corporation, viscosity (aqueous solution

dissolved at 85°C) 3000 to 4000 mPa·s, molecular weight 300,000

Copolyvidone: "VA64" manufactured by BASF, viscosity (5% by weight aqueous solution, 25°C) 5 mPa·s, molecular weight 50,000

[0061] Lactose: lactose hydrate, manufactured by DFE Pharma

[0062] [Method of measuring particle size]

A particle size was measured by a dry dispersion method (unit) using a laser diffraction particle size analyzer ("MASTERSIZER 3000" manufactured by Malvern).

[0063] Reference Examples 1 and 2

(1) Formulation of preparation

The formulations of preparations of Reference Examples 1 and 2 are shown in Table 1 below.

[0064] [Table 1]

Ingredient	Reference Example 1	Reference Example 2
Testosterone A	5 mg	5 mg
Hydroxypropylcellulose H	5 mg	-
Crystalline cellulose	-	5 mg

[0065] (2) Formulation process

Testosterone A was pulverized in a mortar, and then Hydroxypropylcellulose H or Crystalline cellulose was added and mixed thereto. The mixture was filled into a cylindrical device to give a

preparation for administration.

[0066] Incidentally, the cylindrical device is a device that is a needle protector (made of plastic) for a spinal needle ("Terumo Spinal Needle  
5 (registered trademark)" manufactured by Terumo Corporation), the protector having a tip smoothed with a file. In all examples, this container was connected to an oxygen gas spray can (gas for laboratory) to spray a preparation.

10 [0067] (3) Pharmacokinetic evaluation

For each one of aged female dogs (three dogs), a cylindrical device filled with the preparation was inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of  
15 testosterone administered: 5 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after the nasal administration, the blood was collected using a 23G needle and a heparin-treated syringe. The blood collection volume was about 1 ml per point of time.  
20 The collected blood was centrifuged (15000 rpm, 2 minutes, 4°C), and then the plasma was collected and was cryopreserved in a freezer (-20°C or less) until the time of measurement. The plasma testosterone concentration was measured under the following  
25 analysis conditions.

[0068] (HPLC condition)

HPLC: Prominence series (manufactured by

SHIMADZU CORPORATION)

Column: Scherzo SM-C18 (2.0 mm i.d. × 50 mm, 3 μm, manufactured by Imtakt Corporation)

Mobile phase A: 0.1 vol% acetic acid aqueous solution

Mobile phase B: acetonitrile

Column temperature: 40°C

Temperature in autosampler: room temperature

Flow rate: 0.3 ml/min

Injection volume: 10 μL

Gradient condition: shown in the following

Table 2

[0069] [Table 2]

Table 2					
Time (min)	0	4	5	5.1	7.5
Mobile phase A (%)	70	35	0	70	70
Mobile phase B (%)	30	65	100	30	30

15

[0070] (MS condition)

MS/MS: 3200QTRAP (manufactured by AB Sciex)

Ionization method: ESI method (Electrospray Ionization)

Ion polarity: Positive

Measurement ion: shown in the following Table

3

[0071] [Table 3]

Table 3

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	DP (V)	CE (eV)
Testosterone A	289.1	109.1	51	33
Testosterone-d <sub>3</sub>	292.0	109.1	56	35

[0072] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 6.4: manufactured by Certara GK) to calculate a maximum plasma concentration (C<sub>max</sub>), an area under the plasma concentration-time curve to the time of the last quantifiable measurement (or an area under the plasma concentration-time curve to the time of the last measurement at which the measurement value was not less than the quantitative lower limit) (AUC<sub>0-t</sub>), an area under the plasma concentration-time curve extrapolated to infinity (AUC<sub>0-∞</sub>), a time to reach a maximum plasma concentration (T<sub>max</sub>), an elimination half-life (t<sub>1/2</sub>), and a mean residence time in blood (MRT<sub>0-∞</sub>).

[0073] Table 4 shows the pharmacokinetic parameters of the obtained preparations. The plasma testosterone concentration profile is shown in Fig. 1.

[0074] [Table 4]

Table 4

	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-t</sub> (ng·hr/ml)	AUC <sub>0-∞</sub> (ng·hr/ml)	t <sub>1/2</sub> (hr)	MRT <sub>0-∞</sub> (hr)
Reference Example 1	0.4	25.7	109.3	125.4	9.40	9.70
Reference Example 2	0.4	34.4	90.9	92.1	2.67	2.47

[0075] As apparent from the results shown in Table 4 and Fig. 1, the preparation containing the hydroxypropylcellulose as saccharides lowers the maximum plasma concentration ( $C_{max}$ ) and prolongs the elimination half-life ( $t_{1/2}$ ) and the mean residence time in plasma ( $MRT_{0-\infty}$ ) compared with the preparation containing the crystalline cellulose, thus maintaining the plasma testosterone concentration for a longer period of time.

10 [0076] Examples 1 to 2 and Reference Example 3

(1) Formulation of preparation

The formulations of preparations of Examples 1 to 2 and Reference Example 3 are shown in Table 5 below.

15 [0077] [Table 5]

Table 5

Ingredient (average particle size)	Example 1	Example 2	Reference Example 3
Testosterone B classified product (184 $\mu\text{m}$ )	2 mg	-	-
Testosterone B classified product (90.4 $\mu\text{m}$ )	-	2 mg	-
Testosterone B pulverized product (16.2 $\mu\text{m}$ )	-	-	2 mg
Hydroxypropylcellulose H	18 mg	18 mg	18 mg

[0078] (2) Formulation process

To a classified product of Testosterone B or a pulverized product thereof [a classified product which was classified by sieving Testosterone B and of which the volume average particle size was measured

20



after classification (a volume average particle size of 184  $\mu\text{m}$ : Example 1, 90.4  $\mu\text{m}$ : Example 2), and a pulverized product which was obtained by pulverizing Testosterone B in a mortar and of which the volume  
5 average particle size was measured after pulverization (a volume average particle size of 16.2  $\mu\text{m}$ : Reference Example 3)] was added and mixed Hydroxypropylcellulose H. The mixture was filled into a cylindrical device to give a preparation for  
10 administration.

[0079] (3) Pharmacokinetic evaluation

For each one of aged female dogs (three dogs), a cylindrical device filled with the preparation was inserted 1 cm into one of the nasal cavities, and a  
15 single nasal dose was administered (the amount of testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after the nasal administration, the blood was collected using a 23G needle and a syringe. The blood collection volume was  
20 about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm, 10 minutes, 4°C), the plasma was collected and was cryopreserved until the  
25 time of measurement. The plasma testosterone concentration was measured under the following analysis conditions.

[0080] (HPLC condition)

HPLC: Nexera X2 (manufactured by SHIMADZU CORPORATION)

5 Column: Kinetex 2.6  $\mu\text{m}$  EVO C18 (4.6 mm i.d.  $\times$  150 mm, 2.6  $\mu\text{m}$ , manufactured by Phenomenex)

Mobile phase A: 0.05 vol% acetic acid aqueous solution

Mobile phase B: methanol

Column temperature: 45°C

10 Temperature in sample chamber: 10°C

Injection volume: 10  $\mu\text{L}$

Gradient condition: shown in the following

Table 6

[0081] [Table 6]

15

Table 6

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)
0.00	42	58	0.9
0.50	42	58	0.9
11.50	37	63	0.9
11.60	↓	↓	0.9
12.00	1	99	↓
12.50	1	99	1.3
13.50	1	99	1.3
13.51	42	58	1.3
15.50	42	58	1.3
15.51	42	58	0.9
16.00	42	58	0.9

[0082] (MS condition)

MS/MS: Qtrap 6500 (manufactured by AB Sciex)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

7

5 [0083] [Table 7]

Table 7

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	DP (V)	CE (eV)	CXP
Testosterone B	289.2	97.0	80	26	11
Testosterone-d <sub>3</sub>	292.1	96.8	150	27	12

[0084] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 6.4: manufactured by Certara GK) to calculate a maximum plasma concentration ( $C_{max}$ ), an area under the plasma concentration-time curve to 12 hours after administration ( $AUC_{0-12hr}$ ), an area under the plasma concentration-time curve to the time of the last quantifiable measurement (or an area under the plasma concentration-time curve to the time of the last measurement at which the measurement value was not less than the quantitative lower limit) ( $AUC_{0-t}$ ), an area under the plasma concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ), a time to reach a maximum plasma concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-\infty}$ ).

25 [0085] Table 8 shows the pharmacokinetic parameters

results of the obtained preparations. The plasma testosterone concentration profile is shown in Fig. 2.

[0086] [Table 8]

5

Table 8

	$T_{max}$ (hr)	$C_{max}$ (ng/ml)	$AUC_{0-t}$ (ng·hr/ml)	$AUC_{0-\infty}$ (ng·hr/ml)	$t_{1/2}$ (hr)	$MRT_{0-\infty}$ (hr)
Example 1	1.00	4.96	37.80	43.77	8.18	10.75
Example 2	0.33	10.45	54.64	57.82	5.62	6.33
Reference Example 3	0.38	32.25	78.59	79.03	1.26	2.77

[0087] As apparent from the results shown in Table 8 and Fig. 2, Reference Example 3 using the testosterone with less than 50  $\mu m$  shows a maximum plasma concentration ( $C_{max}$ ) exceeding 15 ng/ml that is a criterion by FDA, while Examples 1 to 2 using the testosterone with a large particle size show a maximum plasma concentration ( $C_{max}$ ) maintained to not more than 15 ng/ml that is a criterion by FDA.

10

15 Moreover, Examples 1 to 2 show a low maximum plasma concentration ( $C_{max}$ ), a drastically prolonged elimination half-life of plasma concentration ( $t_{1/2}$ ), and a drastically prolonged mean residence time in plasma ( $MRT_{0-\infty}$ ) compared with Reference Example 3, and thus enable administration twice per day. This leads to an improved medication compliance.

[0088] Moreover, when the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-12hr)}/12$  on the assumption of administration twice per day

(administration every 12 hours), Examples 1 to 2 show an average plasma concentration ( $C_{avg}$ ) within the normal range, 2.01 to 7.5 ng/ml, as shown in Table 9 below.

5 [0089] [Table 9]

No.	Example 1			Example 2		
	1	2	3	1	2	3
$C_{avg}$	2.50	2.36	2.13	4.36	2.86	3.33
Average of $C_{avg}$	2.33			3.51		

[0090] Examples 3 to 5 and Reference Example 4

(1) Formulation of preparation

10 The formulations of preparations of Example 3 to 5 and Reference Example 4 are shown in Table 10 below.

[0091] [Table 10]

Ingredient (average particle size)	Example 3	Example 4	Example 5	Reference Example 4
Testosterone C (classification 273 $\mu\text{m}$ )	2 mg	-	-	-
Testosterone C classified product (131 $\mu\text{m}$ )	-	2 mg	-	-
Testosterone C classified product (70.4 $\mu\text{m}$ )	-	-	2 mg	-
Testosterone C pulverized product (16.2 $\mu\text{m}$ )	-	-	-	2 mg
Lactose	18 mg	18 mg	18 mg	18 mg

15

[0092] (2) Formulation process

To a classified product of Testosterone C or a pulverized product thereof [a classified product which was classified by sieving Testosterone C and of

which the volume average particle size was measured after classification (a volume average particle size of 273  $\mu\text{m}$ : Example 3, 131  $\mu\text{m}$ : Example 4, 70.4  $\mu\text{m}$ : Example 5), and a pulverized product which was  
5 obtained by pulverizing Testosterone C in a mortar and of which the volume average particle size was measured after pulverization (a volume average particle size of 16.2  $\mu\text{m}$ : Reference Example 4)] was added and mixed lactose. The mixture was filled into  
10 a cylindrical device to give a preparation for administration.

[0093] (3) Pharmacokinetic evaluation

For each one of aged male dogs (eight dogs), a cylindrical device filled with the preparation was  
15 inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after the nasal administration, the blood was collected using a 23G  
20 needle and a syringe. The blood collection volume was about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm, 10 minutes, 4°C), the  
25 plasma was collected and was cryopreserved until the time of analysis. The plasma testosterone concentration was measured under the following

analysis conditions.

[0094] (HPLC condition)

HPLC: 2795 (manufactured by Waters corporation)

5 Column: Chromolith Performance RP-18e (3.0 mmi.d. × 100 mm, manufactured by Merck KGaA)

Mobile phase A: 0.005 mol/l ammonium acetate solution

Mobile phase B: methanol

10 Column temperature: 45°C

Temperature in sample chamber: 10°C

Injection volume: 10 µL

Gradient condition: shown in the following

Table 11

15 [0095] [Table 11]

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

[0096] (MS condition)

20 MS/MS: Quattro ultima (manufactured by Waters corporation)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

12

[0097] [Table 12]

Table 12

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	35	15

5

[0098] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 7.0: manufactured by Certara GK) to calculate a maximum plasma

10

concentration ( $C_{max}$ ), an area under the plasma concentration-time curve to 10 hours after administration ( $AUC_{0-10hr}$ ), an area under the plasma concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ), a time to reach a maximum plasma

15

concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-10hr}$  and

$MRT_{0-\infty}$ ). Moreover, the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-10hr)}/10$  on the assumption of administration twice per day

20

(administration every 10 hours).

[0099] Table 13 shows the pharmacokinetic parameters of the obtained preparations. The plasma testosterone concentration profile is shown in Fig. 3. The value represents the mean and the standard deviation (the

25

same applies hereinafter).



[0100] [Table 13]

Table 13

	T (D50)	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-10hr</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	t <sub>1/2</sub> (hr)	MRT <sub>0-10hr</sub> (hr)	MRT <sub>0-∞</sub> (hr)
Example 3	273 μm	0.56± 0.29	3.354± 1.138	10.159± 1.953	17.380± 12.651	4.65± 3.92	3.06± 0.93	7.76± 7.11
Example 4	131 μm	0.69± 0.62	4.121± 1.590	12.634± 5.766	15.789± 6.626	2.97± 1.33	2.79± 0.54	5.17± 2.32
Example 5	70.4 μm	0.81± 0.78	10.419± 3.433	26.289± 8.658	28.486± 8.712	2.30± 0.92	2.26± 0.39	3.18± 1.76
Reference Example 4	16.2 μm	0.31± 0.12	19.639± 6.425	39.614± 13.009	41.080± 12.624	1.52± 0.42	1.77± 0.19	2.20± 0.71

[0101] As apparent from the results shown in Table 13 and Fig. 3, Reference Example 4 using the testosterone with less than 50 μm shows a maximum plasma concentration (C<sub>max</sub>) exceeding 15 ng/ml that is a criterion by FDA, while Examples 3 to 5 using the testosterone with a large particle size show a maximum plasma concentration (C<sub>max</sub>) maintained to not more than 15 ng/ml that is a criterion by FDA.

[0102] Examples 6 to 8

(1) Formulation of preparation

The formulations of preparations of Examples 6 to 8 are shown in Table 14 below.

[0103] [Table 14]

Table 14

Ingredient (average particle size)	Example 6	Example 7	Example 8
Testosterone C classified product (114 μm)	2 mg	2 mg	2 mg
Hydroxypropylcellulose H	18 mg	-	-
Hydroxypropylcellulose M	-	18 mg	-
Hydroxypropylcellulose L	-	-	18 mg

[0104] (2) Formulation process

To a classified product of Testosterone C [a classified product which was classified by sieving Testosterone C and of which the volume average  
5 particle size was measured after classification (a volume average particle size of 114  $\mu\text{m}$ )] was added and mixed hydroxypropylcellulose. The mixture was filled into a cylindrical device to give a preparation for administration.

10 [0105] (3) Pharmacokinetic evaluation

For each one of aged male dogs (eight dogs), a cylindrical device filled with the preparation was inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of  
15 testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after the nasal administration, the blood was collected using a 23G needle and a syringe. The blood collection volume was about 1 ml per point of time. The collected blood was  
20 immediately transferred in a vacuum plasma collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm, 10 minutes, 4°C), the plasma was collected and was cryopreserved until the time of measurement. The plasma testosterone  
25 concentration was measured under the following analysis conditions.

[0106] (HPLC condition)

HPLC: 2795 (manufactured by Waters corporation)

Column: Chromolith Performance RP-18e (3.0 mmi.d. × 100 mm, manufactured by Merck KGaA)

5 Mobile phase A: 0.005 mol/l ammonium acetate aqueous solution

Mobile phase B: methanol

Column temperature: 45°C

Temperature in sample chamber: 10°C

10 Injection volume: 10 µL

Gradient condition: shown in the following

Table 15

[0107] [Table 15]

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

15

[0108] (MS condition)

MS/MS: Quattro ultima (manufactured by Waters corporation)

Ionization method: ESI method

20 Ion polarity: Positive

Measurement ion: shown in the following Table

16

[0109] [Table 16]

Table 16

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	30	15

[0110] The obtained plasma testosterone concentration  
5 was analyzed by a pharmacokinetic analysis software  
(Phoenix WinNonlin Version 7.0: manufactured by  
Certara GK) to calculate a maximum plasma  
concentration (C<sub>max</sub>), an area under the plasma  
concentration-time curve to 10 hours after  
10 administration (AUC<sub>0-10hr</sub>), an area under the plasma  
concentration-time curve extrapolated to infinity  
(AUC<sub>0-∞</sub>), a time to reach a maximum plasma  
concentration (T<sub>max</sub>), an elimination half-life (t<sub>1/2</sub>),  
and a mean residence time in plasma (MRT<sub>0-10hr</sub> and  
15 MRT<sub>0-∞</sub>). Moreover, the average plasma concentration  
(C<sub>avg</sub>) was estimated as AUC(0-10hr)/10 on the  
assumption of administration twice per day  
(administration every 10 hours).

[0111] Table 17 shows the pharmacokinetic parameters  
20 of the obtained preparations. The plasma testosterone  
concentration profile is shown in Fig. 4.

[0112] [Table 17]

Table 17

	Water-soluble polymer	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-10hr</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	t <sub>1/2</sub> (hr)	MRT <sub>0-10hr</sub> (hr)	MRT <sub>0-∞</sub> (hr)	C <sub>avg</sub> (ng/ml)
Example 6	HPC-H	0.66± 0.60	8.576± 3.070	34.318± 10.251	47.809± 13.742	5.33± 2.83	3.50± 0.48	7.74± 3.92	3.432± 1.025
Example 7	HPC-M	0.84± 0.55	7.585± 2.041	32.737± 10.193	55.369± 34.732	7.37± 4.96	3.53± 0.41	10.19± 6.36	3.274± 1.019
Example 8	HPC-L	0.94± 1.26	9.372± 3.489	34.994± 9.333	38.854± 12.438	2.37± 1.02	2.89± 0.52	3.85± 1.37	3.499± 0.933

[0113] As apparent from the results shown in Table 17 and Fig. 4, Examples 6 to 8 using the testosterone having a volume average particle size of 114 μm and the water-soluble polymer in combination show a maximum plasma concentration (C<sub>max</sub>) maintained to not more than 15 ng/ml that is a criterion by FDA and show an average plasma concentration (C<sub>avg</sub>) within the range from 2.01 to 7.5 ng/ml.

[0114] Examples 9 to 12

(1) Formulation of preparation

The formulations of preparations of Examples 9 to 12 are shown in Table 18 below.

[0115] [Table 18]

Table 18

Ingredient (average particle size)	Example 9	Example 10	Example 11	Example 12
Testosterone C classified product (114 μm)	2 mg	2 mg	2 mg	2 mg
Hydroxypropylcellulose M	2 mg	6 mg	10 mg	14 mg
Lactose	16 mg	12 mg	8 mg	4 mg

(2) Formulation process

To a classified product of Testosterone C [a classified product which was classified by sieving

Testosterone C and of which the average particle size was measured after classification (an average particle size of 114  $\mu\text{m}$ )] was added and mixed Hydroxypropylcellulose M and lactose. The mixture was  
5 filled into a cylindrical device to give a preparation for administration.

[0117] (3) Pharmacokinetic evaluation

For each one of aged male dogs (seven dogs), a cylindrical device filled with the preparation was  
10 inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after the nasal  
administration, the blood was collected using a 23G  
15 needle and a heparin-treated syringe. The blood collection volume was about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm,  
20 10 minutes, 4°C), the plasma was collected and was cryopreserved until the time of measurement. The plasma testosterone concentration was measured under the following analysis conditions.

[0118] (HPLC condition)

25 HPLC: 2795 (manufactured by Waters corporation)

Column: Chromolith Performance RP-18e (3.0

mmi.d. × 100 mm, manufactured by Merck KGaA)

Mobile phase A: 0.005 mol/l ammonium acetate solution

Mobile phase B: methanol

5 Column temperature: 45°C

Temperature in sample chamber: 10°C

Injection volume: 10 µL

Gradient condition: shown in the following

Table 19

10 [0119] [Table 19]

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

[0120] (MS condition)

15 MS/MS: Quattro ultima (manufactured by Waters corporation)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

20

20 [0121] [Table 20]

Table 20

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	35	15

[0122] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 7.0: manufactured by Certara GK) to calculate a maximum plasma concentration ( $C_{max}$ ), an area under the plasma concentration-time curve to 10 hours after administration ( $AUC_{0-10hr}$ ), an area under the plasma concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ), a time to reach a maximum plasma concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-10hr}$  and  $MRT_{0-\infty}$ ). Moreover, the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-10hr)}/10$  on the assumption of administration twice per day (administration every 10 hours).

[0123] Table 21 shows the pharmacokinetic parameters of the obtained preparations. The plasma testosterone concentration profile is shown in Fig. 5.

[0124] [Table 21]



Table 21

	Added amount of HPC-M	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-10hr</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	t <sub>1/2</sub> (hr)	MRT <sub>0-10hr</sub> (hr)	MRT <sub>0-∞</sub> (hr)	C <sub>avg</sub> (ng/ml)
Example 9	2 mg	0.43± 0.28	8.983± 4.408	22.656± 11.483	29.327± 12.795	3.77± 1.86	2.69± 0.61	5.62± 3.20	2.266± 1.148
Example 10	6 mg	0.57± 0.31	6.885± 2.236	20.773± 5.245	23.090± 6.902	2.39± 0.97	2.44± 0.40	3.46± 1.35	2.077± 0.525
Example 11	10 mg	0.54± 0.65	7.664± 2.802	24.736± 8.362	29.168± 11.751	2.66± 1.05	2.81± 0.59	4.28± 1.86	2.474± 0.836
Example 12	14 mg	0.50± 0.35	7.563± 2.617	27.928± 8.920	31.688± 10.827	3.07± 0.64	3.28± 0.34	4.56± 0.99	2.793± 0.892

[0125] As apparent from the results shown in Table 21 and Fig. 5, all examples for the added amounts show a maximum plasma concentration (C<sub>max</sub>) maintained to not more than 15 ng/ml that is a criterion by FDA and show an average plasma concentration (C<sub>avg</sub>) within the range from 2.01 to 7.5 ng/ml.

[0126] Examples 13 to 15

10 (1) Formulation of preparation

The formulations of preparations of Examples 13 to 15 are shown in Table 22 below.

[0127] [Table 22]

Table 22

Ingredient (average particle size)	Example 13	Example 14	Example 15
Testosterone C classified product (114 μm)	2 mg	2 mg	2 mg
Hydroxypropylcellulose M	8 mg	-	-
Hydroxypropylcellulose H	-	8 mg	8 mg
Lactose	-	-	10 mg

15

[0128] (2) Formulation process

To a classified product of Testosterone C [a classified product which was classified by sieving

Testosterone C and of which the average particle size was measured after classification (an average particle size of 114  $\mu\text{m}$ )] was added and mixed hydroxypropylcellulose and/or lactose at the  
5 proportion shown in Table 22. The mixture was filled into a cylindrical device to give a preparation for administration.

[0129] (3) Pharmacokinetic evaluation

For each one of aged male dogs (seven dogs), a  
10 cylindrical device filled with the preparation was inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after the nasal  
15 administration, the blood was collected using a 23G needle and a syringe. The blood collection volume was about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly.  
20 After centrifugation (3000 rpm, 10 minutes, 4°C), the plasma was collected and was cryopreserved until the time of measurement. The plasma testosterone concentration was measured under the following analysis conditions.

25 [0130] (HPLC condition)

HPLC: 2795 (manufactured by Waters corporation)

Column: Chromolith Performance RP-18e (3.0  
mmi.d. × 100 mm, manufactured by Merck KGaA)

Mobile phase A: 0.005 mol/l ammonium acetate  
solution

5 Mobile phase B: methanol

Column temperature: 45°C

Temperature in sample chamber: 10°C

Injection volume: 10 µL

Gradient condition: shown in the following

10 Table 23

[0131] [Table 23]

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

[0132] (MS condition)

15 MS/MS: Quattro ultima (manufactured by Waters  
corporation)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

20 24

[0133] [Table 24]

Table 24

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	50	15

[0134] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 7.0: manufactured by Certara GK) to calculate a maximum plasma concentration ( $C_{max}$ ), an area under the plasma concentration-time curve to 10 hours after administration ( $AUC_{0-10hr}$ ), an area under the plasma concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ), a time to reach a maximum plasma concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-10hr}$  and  $MRT_{0-\infty}$ ). Moreover, the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-10hr)}/10$  on the assumption of administration twice per day (administration every 10 hours).

[0135] Table 25 shows the pharmacokinetic parameters of the obtained preparations for administration. The plasma testosterone concentration profile is shown in Fig. 6.

[Table 25]

Table 25

	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-10hr</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	t <sub>1/2</sub> (hr)	MRT <sub>0-10hr</sub> (hr)	MRT <sub>0-∞</sub> (hr)	C <sub>avg</sub> (ng/ml)
Example 13	0.68± 0.64	7.528± 1.488	30.786± 6.932	46.600± 26.594	5.03± 3.41	3.41± 0.54	7.55± 5.30	3.079± 0.693
Example 14	0.57± 0.64	8.870± 3.028	30.160± 7.226	39.179± 13.307	4.09± 1.73	3.27± 0.43	5.98± 3.11	3.016± 0.723
Example 15	0.61± 0.67	9.516± 2.666	34.054± 5.918	38.113± 8.676	3.10± 2.21	2.87± 0.37	4.11± 1.00	3.405± 0.592

[0136] As apparent from the results shown in Table 25 and Fig. 6, all examples show a maximum plasma concentration (C<sub>max</sub>) maintained to not more than 15 ng/ml that is a criterion by FDA and show an average plasma concentration (C<sub>avg</sub>) within the range from 2.01 to 7.5 ng/ml.

[0137] Examples 16 to 21

10 (1) Formulation of preparation

The formulations of preparations of Examples 16 to 21 are shown in Table 26 below.

[0138] [Table 26]

Table 26

Ingredient (average particle size)	Example 16	Example 17	Example 18	Example 19	Example 20	Example 21
Testosterone C classified product (114 μm)	2 mg	2 mg	2 mg	2 mg	2 mg	2 mg
Pregelatinized starch	8 mg	-	-	-	-	-
Carboxyvinyl polymer	-	8 mg	-	-	-	-
Sodium alginate	-	-	8 mg	-	-	-
Hydroxypropylmethylcellulose	-	-	-	8 mg	-	-
Polyvinylpyrrolidone	-	-	-	-	8 mg	-
Xanthan gum	-	-	-	-	-	8 mg

15

[0139] (2) Formulation process

To 2 mg of a classified product of Testosterone C [a classified product which was

classified by sieving Testosterone C and of which the average particle size was measured after classification (an average particle size of 114  $\mu\text{m}$ ) was added and mixed 8 mg of a water-soluble polymer shown in Table 26. The mixture was filled into a cylindrical device to give a preparation for administration.

[0140] (3) Pharmacokinetic evaluation

For each one of aged male dogs (seven dogs), a cylindrical device filled with the preparation was inserted 1 cm into one of the nasal cavities, and a single nasal dose was administered (the amount of testosterone administered: 2 mg). At 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after the nasal administration, the blood was collected using a 23G needle and a syringe. The blood collection volume was about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm, 10 minutes, 4°C), the plasma was collected and was cryopreserved until the time of measurement. The plasma testosterone concentration was measured under the following analysis conditions.

[0141] (HPLC condition)

HPLC: 2795 (manufactured by Waters corporation)

Column: Chromolith Performance RP-18e (3.0  
mmi.d. × 100 mm, manufactured by Merck KGaA)

Mobile phase A: 0.005 mol/l ammonium acetate  
solution

5 Mobile phase B: methanol

Column temperature: 45°C

Temperature in sample chamber: 10°C

Injection volume: 10 µL

Gradient condition: shown in the following

10 Table 27

[0142] [Table 27]

Table 27

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

[0143] (MS condition)

15 MS/MS: Quattro ultima (manufactured by Waters  
corporation)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

20 28

[0144] [Table 28]

Table 28

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	35	15

[0145] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software (Phoenix WinNonlin Version 7.0: manufactured by Certara GK) to calculate a maximum plasma concentration ( $C_{max}$ ), an area under the plasma concentration-time curve to 10 hours after administration ( $AUC_{0-10hr}$ ), an area under the plasma concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ), a time to reach a maximum plasma concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-10hr}$  and  $MRT_{0-\infty}$ ). Moreover, the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-10hr)}/10$  on the assumption of administration twice per day (administration every 10 hours).

[0146] Table 29 shows the pharmacokinetic parameters of the obtained preparations for administration. The plasma testosterone concentration profile is shown in Fig. 7.

[0147] [Table 29]



Table 29

	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/ml)	AUC <sub>0-10hr</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	t <sub>1/2</sub> (hr)	MRT <sub>0-10hr</sub> (hr)	MRT <sub>0-∞</sub> (hr)	C <sub>avg</sub> (ng/ml)
Example 16	0.82± 0.81	8.223± 3.444	26.705± 9.659	33.250± 20.286	4.07± 5.05	2.51± 0.30	5.04± 4.39	2.671± 0.966
Example 17	0.96± 0.74	8.740± 2.329	30.036± 6.828	45.663± 14.155	7.72± 10.16	3.13± 0.60	10.86± 12.09	3.004± 0.683
Example 18	0.54± 0.65	8.430± 2.068	29.675± 11.078	36.124± 17.529	3.56± 1.06	3.04± 0.48	4.90± 1.54	2.968± 1.108
Example 19	0.29± 0.09	8.490± 3.198	35.202± 12.148	46.203± 16.777	4.25± 1.30	3.41± 0.66	6.40± 2.04	3.520± 1.215
Example 20	0.86± 0.83	7.078± 2.064	22.590± 8.900	31.208± 14.526	4.64± 3.34	2.65± 0.73	6.37± 4.13	2.259± 0.890
Example 21	0.64± 0.66	9.789± 4.048	35.424± 17.330	41.866± 24.633	2.78± 0.81	2.93± 0.45	3.95± 1.36	3.542± 1.733

[0148] As apparent from the results shown in Table 29 and Fig. 7, all examples show a maximum plasma concentration (C<sub>max</sub>) maintained to not more than 15 ng/ml that is a criterion by FDA and show an average plasma concentration (C<sub>avg</sub>) within the range from 2.01 to 7.5 ng/ml.

[0149] Examples 22 to 27

10 (1) Formulation of preparation

The formulations of preparations of Examples 22 to 27 are shown in Table 30 below.

[0150] [Table 30]

Table 30

Ingredient (average particle size)	Example 22	Example 23	Example 24	Example 25	Example 26	Example 27
Testosterone C classified product (114 $\mu\text{m}$ )	2 mg	2 mg	2 mg	2 mg	2 mg	2 mg
Polyethylene glycol	8 mg	-	-	-	-	-
Polyvinyl alcohol	-	8 mg	-	-	-	-
Chitosan	-	-	8 mg	-	-	-
Carmellose sodium	-	-	-	8 mg	-	-
Locust bean gum	-	-	-	-	8 mg	-
Copolyvidone	-	-	-	-	-	8 mg

[0151] (2) Formulation process

To 2 mg of a pulverized product of  
5 Testosterone C [a classified product which was  
obtained by pulverizing Testosterone C in a mortar  
and of which the volume average particle size was  
measured after pulverization (a volume average  
particle size of 114  $\mu\text{m}$ )] was added and mixed 8 mg of  
10 a water-soluble polymer shown in Table 30. The  
mixture was filled into a cylindrical device to give  
a preparation for administration.

[0152] (3) Pharmacokinetic evaluation

For each one of aged male dogs (seven dogs), a  
15 cylindrical device filled with the preparation was  
inserted 1 cm into one of the nasal cavities, and a  
single nasal dose was administered (the amount of  
testosterone administered: 2 mg). At 0.25, 0.5, 1, 2,  
4, 6, 8, 10, and 24 hours after the nasal  
20 administration, the blood was collected using a 23G  
needle and a syringe. The blood collection volume was

about 1 ml per point of time. The collected blood was immediately transferred in a vacuum blood collection tube with heparin sodium and was stirred lightly. After centrifugation (3000 rpm, 10 minutes, 4°C), the plasma was collected and was cryopreserved until the time of measurement. The plasma testosterone concentration was measured under the following analysis conditions.

[0153] (HPLC condition)

10 HPLC: 2795 (manufactured by Waters corporation)

Column: Chromolith Performance RP-18e (3.0 mmi.d. × 100 mm, manufactured by Merck KGaA)

15 Mobile phase A: 0.005 mol/l ammonium acetate solution

Mobile phase B: methanol

Column temperature: 45°C

Temperature in sample chamber: 10°C

Injection volume: 10 µL

20 Gradient condition: shown in the following Table 31

[0154] [Table 31]

Table 31

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Curve
0.00	40	60	0.40	1
1.00	40	60	0.40	1
4.00	20	80	0.40	6
6.00	10	90	0.40	6
6.50	5	95	0.90	11
8.50	40	60	0.40	11

[0155] (MS condition)

MS/MS: Quattro ultima (manufactured by Waters  
5 corporation)

Ionization method: ESI method

Ion polarity: Positive

Measurement ion: shown in the following Table

32

10 [0156] [Table 32]

Table 32

Measuring object	Precursor ion [M+H] <sup>+</sup> (m/z)	Product ion (m/z)	Cone (V)	Col (eV)
Testosterone C	289.3	97.0	50	18
Testosterone-d <sub>3</sub>	292.3	97.0	35	15

[0157] The obtained plasma testosterone concentration was analyzed by a pharmacokinetic analysis software  
15 (Phoenix WinNonlin Version 7.0: manufactured by Certara GK) to calculate a maximum plasma concentration (C<sub>max</sub>), an area under the plasma concentration-time curve to 10 hours after administration (AUC<sub>0-10hr</sub>), an area under the plasma  
20 concentration-time curve extrapolated to infinity

( $AUC_{0-\infty}$ ), a time to reach a maximum plasma concentration ( $T_{max}$ ), an elimination half-life ( $t_{1/2}$ ), and a mean residence time in plasma ( $MRT_{0-10hr}$  and  $MRT_{0-\infty}$ ). Moreover, the average plasma concentration ( $C_{avg}$ ) was estimated as  $AUC_{(0-10hr)}/10$  on the assumption of administration twice per day (administration every 10 hours).

[0158] Table 33 shows the pharmacokinetic parameters of the obtained preparations for administration. The plasma testosterone concentration profile is shown in Fig. 8.

[0159] [Table 33]

	$T_{max}$ (hr)	$C_{max}$ (ng/ml)	$AUC_{0-10hr}$ (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	$t_{1/2}$ (hr)	$MRT_{0-10hr}$ (hr)	$MRT_{0-\infty}$ (hr)	$C_{avg}$ (ng/ml)
Example 22	0.64±	6.858±	20.259±	38.036±	8.75±	3.11±	13.05±	2.026±
	0.66	2.321	4.088	12.818	7.64	0.61	10.21	0.409
Example 23	0.39±	6.278±	20.113±	33.687±	6.35±	3.15±	9.86±	2.011±
	0.28	2.156	3.929	14.263	5.44	0.66	8.02	0.393
Example 24	0.54±	8.982±	29.488±	35.591±	3.07±	3.11±	4.90±	2.949±
	0.65	2.981	5.917	10.257	0.70	0.44	1.40	0.592
Example 25	0.71±	7.649±	24.258±	30.129±	3.42±	2.60±	5.07±	2.426±
	0.67	2.372	7.218	9.260	2.11	0.52	2.42	0.722
Example 26	0.64±	6.814±	23.934±	29.718±	3.16±	3.10±	5.29±	2.393±
	0.66	2.404	5.193	7.382	0.88	0.37	2.11	0.519
Example 27	0.86±	7.579±	19.723±	24.034±	5.08±	2.72±	6.37±	1.972±
	0.83	2.486	4.819	5.097	4.61	0.61	4.54	0.482

[0160] As apparent from the results shown in Table 33 and Fig. 8, all examples show a maximum plasma concentration ( $C_{max}$ ) maintained to not more than 15

ng/ml that is a criterion by FDA, and Examples 22 to 26 show an average plasma concentration ( $C_{avg}$ ) within the range from 2.01 to 7.5 ng/ml. Whereas, Example 27 shows an average plasma concentration ( $C_{avg}$ ) less than 2.01 ng/ml, probably because of a low viscosity of the water-soluble polymer.

#### INDUSTRIAL APPLICABILITY

[0161] The powder preparation for nasal administration of the present invention can be used as various therapeutic agents utilizing the steroid hormones (e.g., a therapeutic agent for late-onset hypogonadism, an anticancer agent, a therapeutic agent for menopausal disorder, a therapeutic agent for osteoporosis, a therapeutic agent for chronic kidney disease, a therapeutic agent for infertility, a therapeutic agent for menstrual disorder, a contraceptive, a therapeutic agent for functional uterine bleeding, and a therapeutic agent for endometriosis). In particular, the powder preparation containing testosterone as the steroid hormones can be used effectively as a therapeutic agent for late-onset hypogonadism.

[0162] It is to be understood that, if any prior art  
publication is referred to herein, such reference  
does not constitute an admission that the publication  
forms a part of the common general knowledge in the  
5 art, in Australia or any other country.

## CLAIMS

[Claim 1] A powder preparation for nasal administration, containing steroid hormones being in a particulate form and having an average particle size of 50 to 300  $\mu\text{m}$  as an active ingredient,

wherein the powder preparation controls an average plasma concentration ( $C_{\text{avg}}$ ) of the steroid hormones to 2 to 7.5 ng/ml.

[Claim 2] The powder preparation for nasal administration according to claim 1, which further contains a water-soluble polymer.

[Claim 3] The powder preparation for nasal administration according to claim 2, wherein the water-soluble polymer contains water-soluble polysaccharides.

[Claim 4] The powder preparation for nasal administration according to claim 3, wherein the water-soluble polysaccharides contain a cellulose having a hydroxyalkyl group.

[Claim 5] The powder preparation for nasal administration according to any one of claims 2 to 4, wherein the water-soluble polymer is free from a crystalline cellulose and a carboxymethylcellulose sodium.



[Claim 6] The powder preparation for nasal administration according to any one of claims 2 to 5, wherein the water-soluble polymer is in a particulate form.

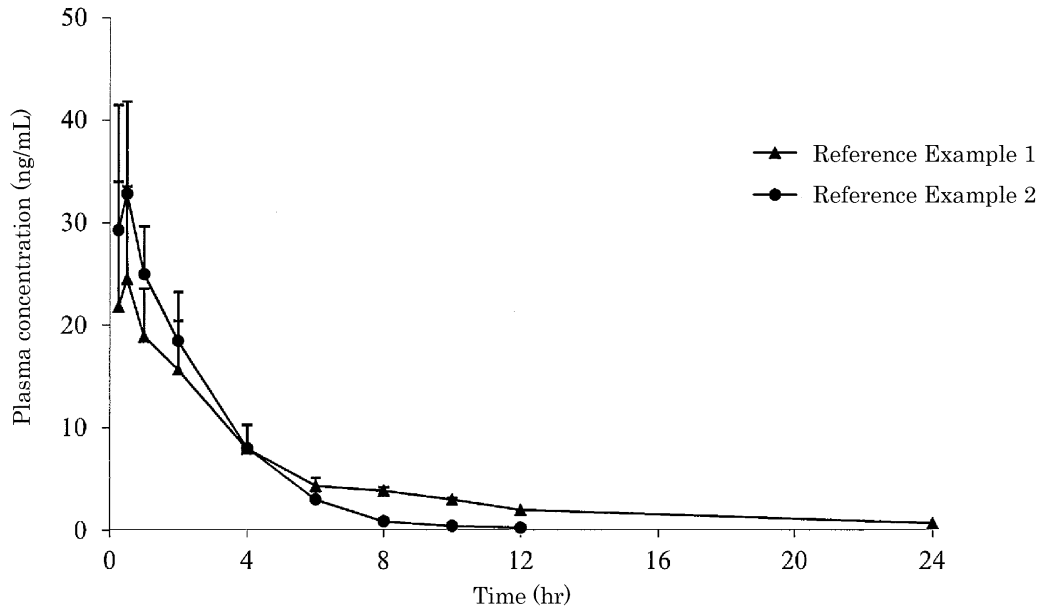
[Claim 7] The powder preparation for nasal administration according to any one of claims 1 to 6, wherein the steroid hormones are a male hormone.

[Claim 8] The powder preparation for nasal administration according to claim 7, wherein the male hormone is at least one selected from testosterone, a C<sub>1-4</sub>alkyltestosterone, a C<sub>1-18</sub>alkanoic acid ester of testosterone, a cycloalkanoic acid ester of testosterone, dihydrotestosterone and dehydroepiandrosterone.

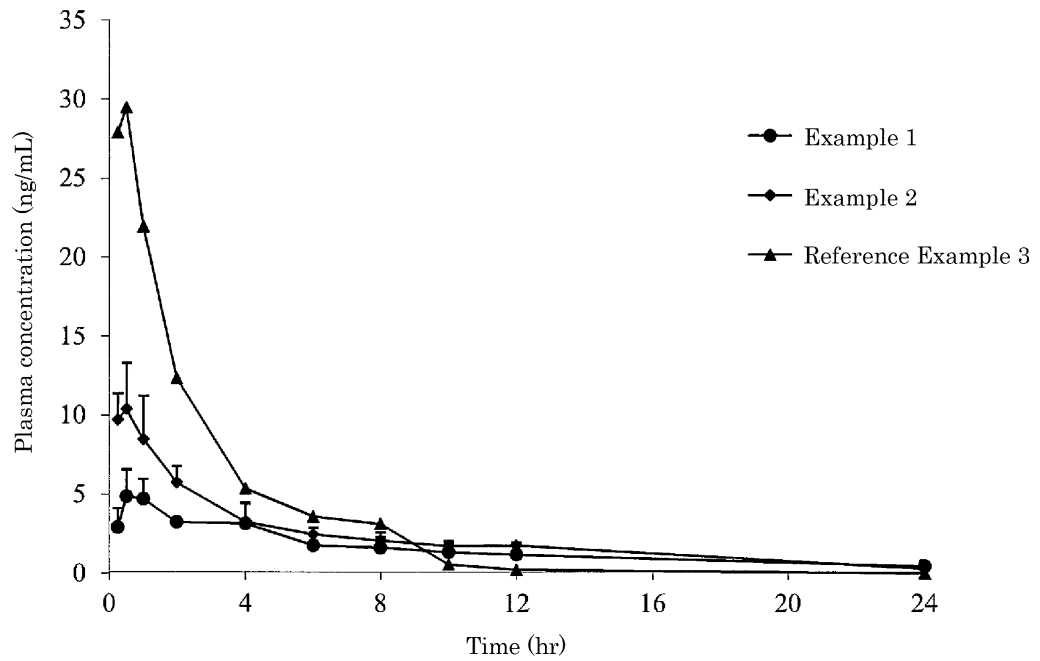
[Claim 9] The powder preparation for nasal administration according to any one of claims 2 to 8, wherein a ratio of the water-soluble polymer is 1 to 50 parts by weight relative to 1 part by weight of the steroid hormones.

[Claim 10] The powder preparation for nasal administration according to any one of claims 1 to 9, which controls a maximum plasma concentration (C<sub>max</sub>) of the steroid hormones to not more than 15 ng/ml.

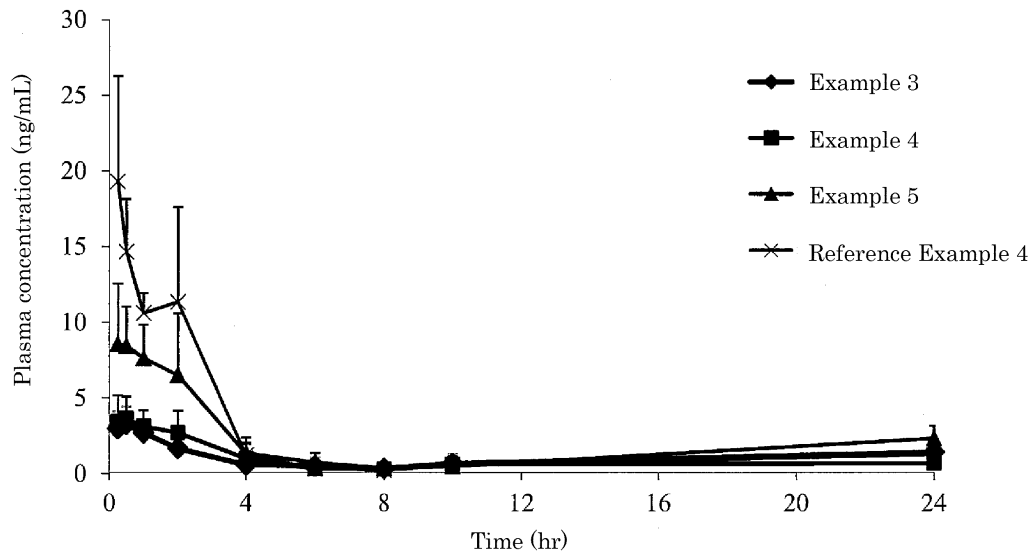
[Fig. 1]



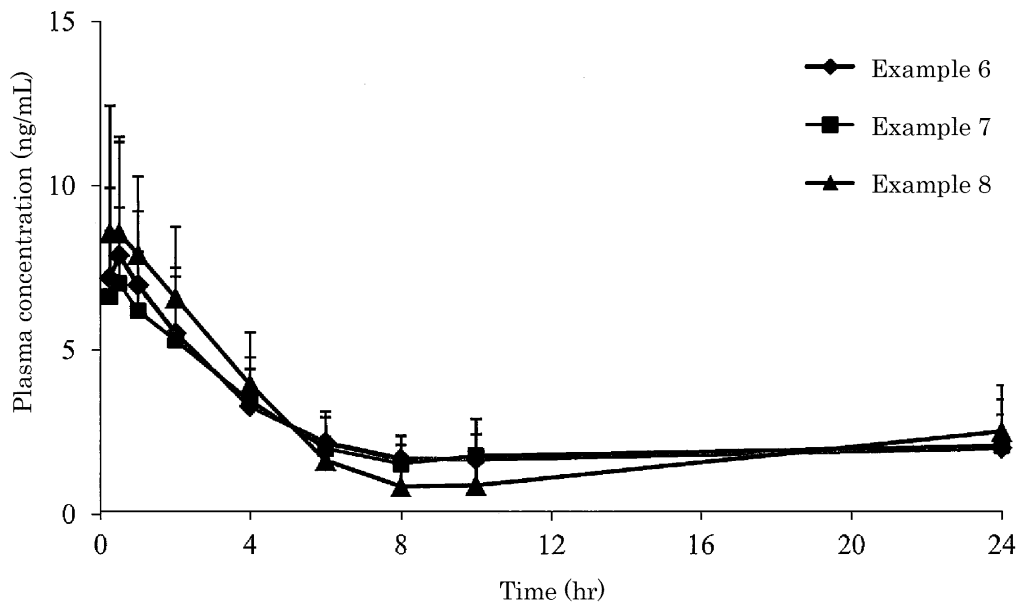
[Fig. 2]



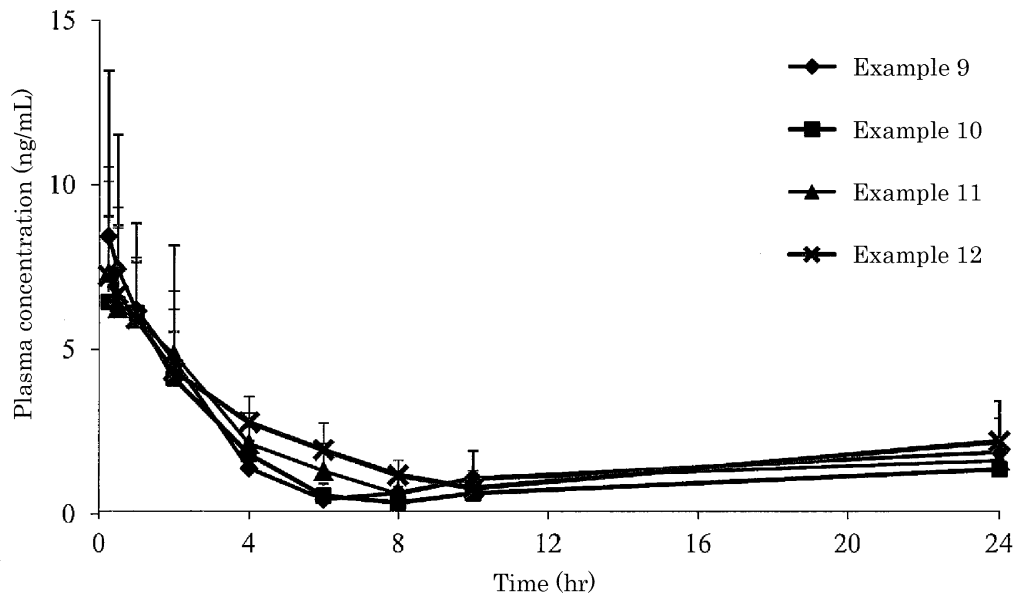
[Fig. 3]



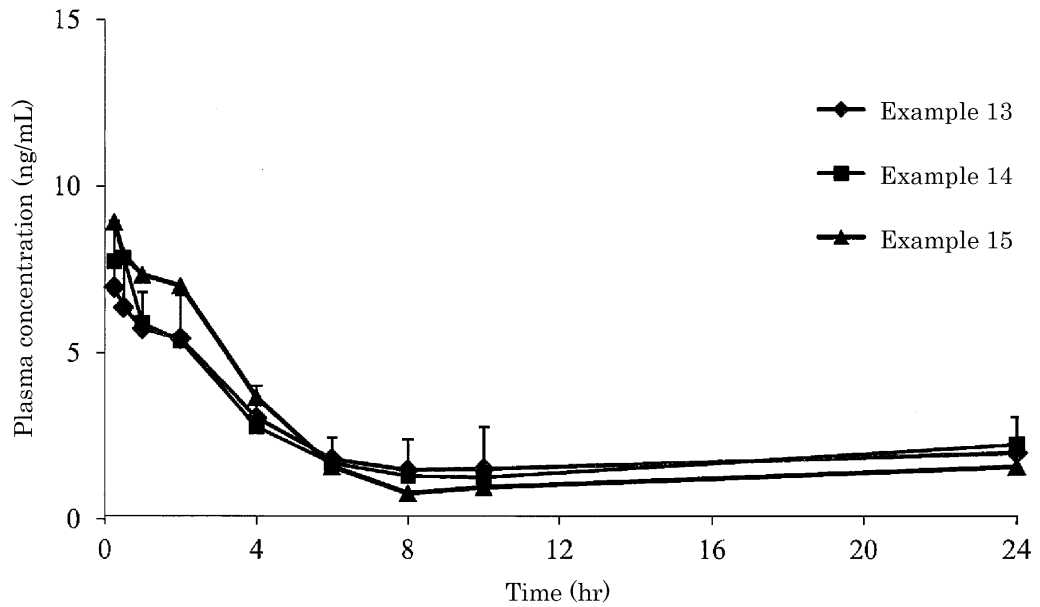
[Fig. 4]



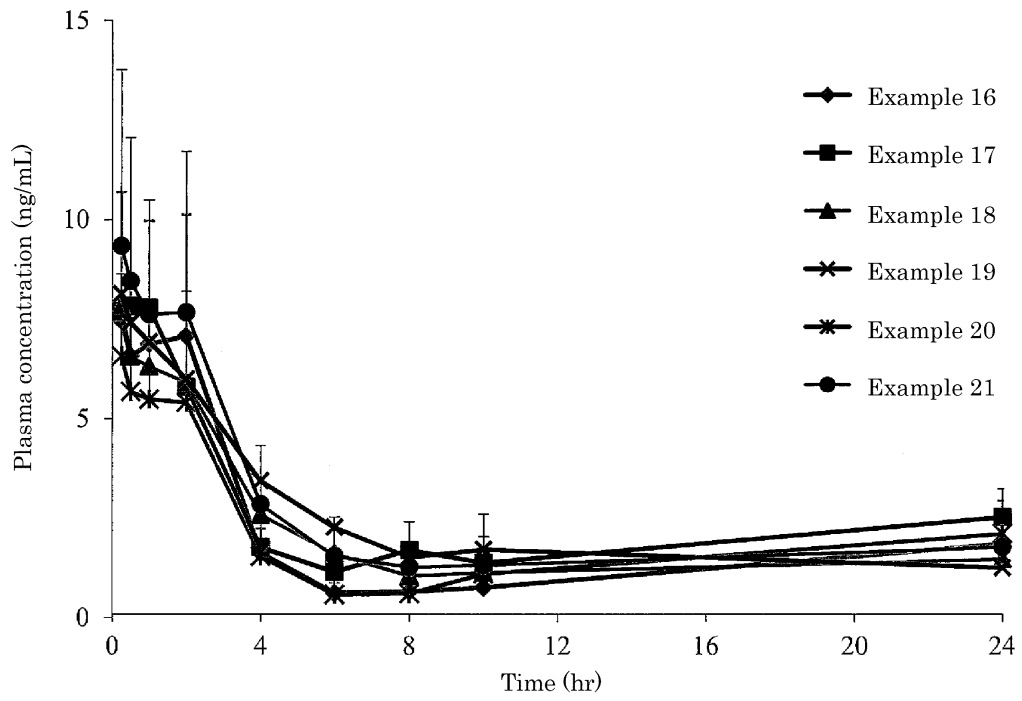
[Fig. 5]



[Fig. 6]



[Fig. 7]



[Fig. 8]

