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(54) Title: EXTRACT AND FORMULATION INCLUDING EXTRACT

(54) 発明の名称: 抽出物及び該抽出物を含有する製剤

(57) Abstract: The objective of the present invention is to provide an extract from inflamed rabbit skin inoculated with vaccinia virus and formulations, etc. including the extract which have greater uniformity in quality. By using the amount of N-acetyl neuraminic acid contained in the extract from inflamed rabbit skin inoculated with vaccinia virus and the formulations including the extract as an index, greater uniformity of the extract and the formulations can be ensured for each production lot. The extract from inflamed rabbit skin inoculated with vaccinia virus and the formulations including the extract, which are produced in this manner so as to have greater uniformity, are extremely useful with effectiveness and safety more rigorously secured.

(57) 要約: 本発明の目的は、品質のより安定したワクシニアウイルス接種ウサギ炎症皮膚抽出物及び当該抽出物を含有する製剤等を提供することにある。ワクシニアウイルス接種ウサギ炎症皮膚抽出物及び当該抽出物を含有する製剤に含有されるN-アセチルノイタミン酸の量を指標とすることにより、当該抽出物及び製剤の製造ロット毎の品質をより安定的なものとして確保することができる。そのような方法で品質がより安定なものとなったワクシニアウイルス接種ウサギ炎症皮膚抽出物及び当該抽出物を含有する製剤は、その有効性及び安全性もより厳格に担保されたものとなり、非常に有用性の高いものである。

## DESCRIPTION EXTRACT AND FORMULATION INCLUDING EXTRACT

### [Technical Field]

[0001]

The present invention relates to an extract from inflamed skins of rabbits inoculated with vaccinia virus wherein the quality is more stabilized by confirming by such a means of tests or inspections that it contains a predetermined amount of N-acetylneuraminic acid and also to a preparation, etc. in which the extract is an active ingredient.

### [Background Art]

[0002]

Drug is approved for its manufacture and distribution only after its quality is warranted. In Japan, that is stipulated in Article 14 of the Pharmaceutical Affairs Law. In view of the characteristics of a drug, such a management is basically same in other countries as well. The reason why warranty of the quality is important for a drug as such is that the quality guarantees efficacy and safety of a drug. Conversely, with regard to a drug for which no quality is warranted, neither efficacy nor safety is guaranteed therefore whereby such a one is not competent as a drug.

[0003]

In Japan, a material substance being used for the production of a drug and able to be an active ingredient of a drug is called a "drug substance". The same as in a drug, a drug substance is also necessary that its quality is warranted. That is because the quality of a drug depends upon the quality of a drug substance. Incidentally, according to Japanese laws and regulations related to pharmaceutical affairs, a drug substance is stipulated as a drug which is exclusively used for the manufacture of other drug and, in

terms of the definition therefore, a drug substance is covered by a drug. However, in the present application, a medicament and a drug substance may be sometimes separately referred to for the sake of convenience and, in such a case, the meaning of a drug shall exclude a drug substance.

[0004]

It is general that, when a drug or a drug substance is manufactured by a predetermined manufacturing method, that having a predetermined quality is manufactured. Accordingly, in order to maintain the quality of a drug or a drug substance, manufacture control is also important. For such a purpose, there has been stipulated "Ministerial Ordinance concerning the Standards for Manufacture Control and Quality Control of Drugs and Quasi-Drugs" in Japan. This ministerial ordinance is called GMP (abbreviation of Good Manufacturing Practice) in Japan as well. In GMP, "manufacture control" and "quality control" of drugs, etc. are stipulated. In "manufacture control", there is adopted such a way of thinking that the quality is maintained by controlling the material preparation and the manufacturing steps from the initial to the final stages thereof. "Quality control" has been mainly conducted by means of confirmation of the tests and inspections conducted after the manufacture whether the manufactured drug or drug substance actually has predetermined quality standards. When the result of the tests and inspections does not satisfy the previously stipulated standards, then shipment, distribution, use, etc. of the drug and drug substance are not allowed. As such, the quality of drugs and drug substances are controlled by means of the manufacture control and the quality control.

[0005]

An extract from inflamed skins of rabbits inoculated with vaccinia virus (hereinafter, it will be sometimes referred to as "the present extract") is an extract containing a non-proteinous active substance extracted and

separated from the inflamed skin tissues of rabbits by the inoculation of vaccinia virus. Although the present extract is liquid in an extracted state, it is also possible to make into a solid by means of drying.

[0006]

As will be mentioned later, a preparation containing the present extract as an active ingredient (hereinafter, it will be sometimes called "the present preparation") is very useful as a drug. Since the present extract is an active ingredient of the present preparation in that case, the present extract is a drug substance of the present preparation. In a specific product as the present preparation which is manufactured and distributed by the applicant, there is "a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus" (trade name: NEUROTROPIN [registered trade mark]; hereinafter, it will be referred to as "NTP preparation"). In NTP preparation, there are injection (hereinafter, it will be referred to as "NTP injection") and tablet (hereinafter, it will be referred to as "NTP tablet") and both belong to an ethical drug. An extract from inflamed skins of rabbits inoculated with vaccinia virus (hereinafter, it will be referred to as "NTP extract") which is an active ingredient of NTP preparation is a drug substance of NTP preparation. NTP extract is covered by the present extract while NTP preparation (NTP injection and NTP tablet) is covered by the present preparation.

[0007]

Indications of NTP injection are "low back pain, cervicobrachial syndrome, symptomatic neuralgia, itchiness accompanied by skin diseases (eczema, dermatitis, urticaria), allergic rhinitis and sequelae of subacute myelo-optico-neuropathy (SMON) such as coldness, paresthesia and pain". Indications of NTP tablet are "postherpetic neuralgia, low back pain, cervicobrachial syndrome, periarthritis scapulohumeralis and osteoarthritis". NTP preparation has been created and developed as a drug by the applicant.

NTP preparation has been appreciated for its excellent advantage for efficacy and safety, sold for many years and established a firm position in the Japanese pharmaceutical market. At present, NTP preparation is being exported to China and sold under a trade name of “神経妥楽平 / NEUROTROPIN”. Indications of NTP preparation in China are the same as those in Japan.

[0008]

As such, the present preparation is very useful as a drug and the present extract is also very useful as a drug substance for the present preparation. However, as mentioned already, the present extract is extracted and separated from the inflamed skin tissues of rabbit by the inoculation of vaccinia virus. Therefore, the present extract contains quite a lot of substances (components) and the present preparation manufactured using the present extract also contains quite a lot of substances (components). Accordingly, it is a very important matter how to control the quality of the present extract and the present preparation in a stable one.

[0009]

Many of drugs are the preparations containing one or two to three substances (components) at the largest as an active ingredient and, usually, such substances are the chemically synthesized compounds. Therefore, when the content of the compound(s) in said preparation is measured and a predetermined content is contained, the quality of said preparation in view of the content of the active ingredient(s) is guaranteed. However, the present extract is an extract from inflamed skin tissues of rabbits by the inoculation of vaccinia virus and contains quite many kinds of substances. It goes without saying that the present preparation where the present extract is an active ingredient also contains quite many kinds of substances. Thus, in the present extract and the present preparation, specific one or several kind(s) of substance(s) is/are not the active ingredient(s) as such

whereby it is not possible to conduct a quality control as in the case of conventional drug where active ingredient(s) is/are specified as substance(s). Therefore, quantitative determination for active ingredient(s) of the present extract and the present preparation manufactured by the applicant or of NTP extract and NTP preparation is being carried out by such a method where the biological activity (titer) thereof is measured.

[0010]

Said method is a biological test method where an analgesic coefficient is determined using SART-stressed (repeated cold stressed) animals which are chronic stressed animals showing a lowered pain threshold than normal animals (*Folia Pharmacologica Japonica*, vol. 72, no. 5, pages 573 to 584, 1976). In accordance with the method in this literature, the analgesic coefficient is determined by conducting an analgesic test according to a Randall-Selitto method using SART-stressed (repeated cold stressed) animals. In this method, an analgesic effect is measured using a pressure weight as an index where the pressure stimulation is applied to the tail of mouse and the mouse shows an escape reaction. An analgesic coefficient is a value where the pressure weight measured after administration of a drug is divided by the value before the administration. In NTP extract and NTP preparation, the case where an analgesic coefficient showed more than a predetermined value defined by the applicant is judged to be positive for an analgesic effect and the rate of the numbers of animals judged to be positive is determined and used as an analgesic efficacy rate (%). This value is used and ED<sub>50</sub> value is determined from the result upon measurement of standard preparation diluted to various concentrations. "Neurotropin Unit (NU)" which is a unit of biological activity (titer) using for NTP preparation by the applicant is defined that the activity of 1 mg of the present extract where the ED<sub>50</sub> value is the dose of 100 mg/kg is 1 Neurotropin unit. ED<sub>50</sub> value for each NTP preparation is measured and compared with that of the standard

preparation whereupon an analgesic activity (active ingredient content) is quantitatively determined. Hereinafter in the present application, the word "unit" is used as a measure of the active ingredient content (titer) of the present extract and the present preparation and it is substantially in the same meaning as "Neurotropin Unit" used for NTP extract and NTP preparation.

[0011]

In the meanwhile, it is stipulated that the present extract and the present preparation or NTP extract and NTP preparation manufactured by the applicant are to be subjected to the following plural identity tests in addition to the above-mentioned quantification of the analgesic activity and should conform to them. Thus, with regard to the present extract and the present preparation manufactured by the applicant, not only the above-mentioned biological activity (titer) is used for guaranteeing the appropriateness of the manufactured lots but also the following plural identity tests are carried out and adaptation thereto is used as a necessary condition for use and shipment of the present extract and the present preparation.

- Identity test of amino acids by a liquid chromatographic method
- Identity test of ultraviolet absorbing substances by

Ultraviolet-visible Spectrophotometry

- Identity test of phosphorus by a color reaction method
- Identity test of nucleic acid base by a liquid chromatographic method
- Identity test of inhibitory action for the production of kallikrein-like substance by an *in vitro* test method

[0012]

However, even when such tests are carried out, it is not always true that the amino acids, ultraviolet absorbing substances, phosphorus, nucleic

acid bases, etc. which are objects of the tests are the crucial effective ingredients of the present extract and the present preparation. Moreover, in those tests, the identity test for amino acids, ultraviolet absorbing substances, phosphorus and nucleic acid bases is a qualitative test which merely confirms the presence of amino acids, etc. which are objects of the tests and does not check how much amount is contained therein. Even under such circumstances however, those tests are still obligatory for the applicant in order to receive an approval for the manufacture of drugs from the government as a means for reducing the variations in the quality among the manufacturing lots and for guaranteeing the uniform quality in both of the present extract and the present preparation where the effective ingredients are unidentified.

[0013]

Generally speaking, it is advantageous for persons who manufacture an extract from living organisms such as animals and plants or a preparation containing said extract as an active ingredient if and when the standards to which said preparation should conform are small in numbers since time, labor and cost for the test and the inspection are saved and possibility where the manufactured product does not conform to the standard is low. However, in view of guaranteeing the quality of the above extract and preparation, it is desirable that the standard as such is stipulated more strictly. Under such circumstances, the applicant has extensively investigated the new appropriate standard to which the present extract and the present preparation manufactured by the applicant should conform whereupon the present invention has been achieved. Thus, the amount of N-acetylneuraminic acid contained in the present extract and the present preparation is measured and, when the amount is within a predetermined range, it is treated that the present extract and the present preparation as such are appropriately manufactured or, in other words, their

use and shipment are permitted. As a result, the variations among manufacturing lots of the present extract and the present preparation are further reduced and the quality thereof becomes more stable. Further, as a result, efficacy and safety of the present extract and the present preparation are guaranteed more strictly. If and when there is the present extract or the present preparation wherein the amount of N-acetylneuraminic acid is out of the stipulated range, it is treated as an adulterated product (a substandard product) whereby it is possible to control the quality of the present extract and the present preparation in a more stable manner. Thus, it is now possible to stipulate an autonomous or a public standard concerning the amount of N-acetylneuraminic acid contained in the present extract or the present preparation. In the meanwhile, the applicant has not stipulated a standard for the amount of N-acetylneuraminic acid contained in an NTP extract and an NTP preparation manufactured up to now and has not conducted such an act that the amount is measured and that, after confirming the measured amount is within a predetermined range, the corresponding NTP extract and NTP preparation are used, shipped, etc.

[0014]

As to the documents which disclose the present extract or the present preparation, Patent Documents 1 to 3 are available. Those documents disclose the content of amino acids and nucleic acid bases in the present extract or the present preparation. Further, with regard to the present extract or the present preparation, there is a disclosure for the content of silicons in Patent Document 4. However, in those Patent Documents 1 to 4, there is no disclosure at all how much a specific substance which is N-acetylneuraminic acid is contained in the present extract or the present preparation. It further goes without saying that, in those documents, there is neither disclosure nor suggestion at all to adopt the content of N-acetylneuraminic acid in the present extract or the present preparation as

an index for the quality control of the present extract or the present preparation in more a stable manner.

[Prior Art Documents]

[Patent Documents]

[0015]

Patent Document 1: Chinese Patent Laid-Open No. CN 1205233A

Patent Document 2: International Publication No. WO 2004/060381

Patent Document 3: Chinese Patent Laid-Open No. CN 1613305A

Patent Document 4: Japanese Patent Laid-Open No. Patent  
07/097336

[Summary of the Invention]

[Problems to be solved by the invention]

[0016]

As mentioned above, no single substance is identified as an active ingredient in the present extract and the present preparation. Therefore, quality of the present extract and the present preparation is guaranteed by a titer test according to a biological test determining the analgesic coefficient using SART-stressed mice, plural identity tests, etc. Even by the means as such, it is still unavoidable that various components contained in the present extract and the present preparation permissibly vary for each manufacturing lot. However, since the present preparation is used as a drug for treating the diseases, it is desirable that the quality thereof is to be as constant as possible. It is very meaningful that the quality of the present extract and the present preparation is warranted to be more stable since it makes efficacy and safety of the present extract and the present preparation more constant.

[Means for Solving the Problems]

[0017]

The present invention is to warrant the quality of the present extract and the present preparation using the amount of N-acetylneuraminic acid contained in the present extract and the present preparation as an index. Thus, based on the above, the present invention is to provide the present extract, the present preparation, etc. which are surely manufactured in an appropriate manner by means of confirmation of containing a stipulated amount of N-acetylneuraminic acid.

[Advantages of the Invention]

[0018]

The present extract and the present preparation in accordance with the present invention are such ones which contain predetermined amounts of N-acetylneuraminic acid. As a result thereof, it is possible to treat that the present extract and the present preparation in accordance with the present invention are appropriately manufactured. The present invention contributes in such a matter that the quality of the present extract and the present preparation is warranted in a more stable manner and efficacy and safety of the present extract and the present preparation become more constant.

[Mode for Carrying Out the Invention]

[0019]

N-Acetylneuraminic acid is a kind of sialic acid. Sialic acid is a generic name for acyl derivatives of neuraminic acid. Although there are many kinds of sialic acids in nature, N-acetylneuraminic acid is the most abundant one among them and the next abundant one is N-glycolylneuraminic acid. Sialic acid is widely distributed in living bodies

as a constituting component of glycoprotein, glycolipid, glycopeptide, etc. and it is particularly present on the cell membrane surface of animals or bacteria and plays an important role of biological functions such as participation in specific recognition mechanism of cells. Sialic acid is also considered to be important in medical and pharmaceutical viewpoints as a substance participating in cancer, inflammation, immune, viral infection, cell differentiation, hormone receptors, etc. Various studies have been conducted for sialic acid-containing biological components and also for sialic acid and derivatives thereof.

[0020]

With regard to said sialic acid and particularly to N-acetylneuraminic acid, the applicant has quite surprisingly found that said substance has an analgesic action and particularly an effect for non-inflammatory pain diseases such as neuropathic pain disease and, on the basis of such a finding, the applicant obtained patents (Japanese Patent No. 4005115 and Chinese Patent Publication No. CN 101600438B). Since the characteristic of NTP preparation manufactured and distributed by the applicant is also a treating effect for neuropathic pain disease, etc., N-acetylneuraminic acid is a rational substance (component) as an index for a strict control of the quality of the present extract and the present preparation in a more stable manner.

[0021]

Next, a method for manufacturing the present extract and the present preparation will be illustrated.

The present extract can be prepared by such a manner that inflamed skin tissues of rabbits by the inoculation of vaccinia virus are collected, crushed and processed by adding an extraction solvent thereto, tissue fragments are removed therefrom, a treatment for removal of protein (deproteinization) is carried out, the resulting one is adsorbed with an

adsorbent under an acidic condition and then an active ingredient is eluted under a basic condition therefrom.

[0022]

Vaccinia virus used herein may be in any strain. Examples thereof include Lister strain, Dairen strain, Ikeda strain, EM-63 strain and New York City Board of Health strain.

[0023]

With regard to a rabbit, any rabbit may be used so far as it belongs to *Lagomorpha*. Examples thereof include *Oryctolagus cuniculus*, domestic rabbit (domesticated *Oryctolagus cuniculus*), hare (Japanese hare), mouse hare and snowshoe hare. Among them, it is appropriate to use domestic rabbit. In Japan, there is family rabbit called "Kato" which has been bred since old time and frequently used as livestock or experimental animal and it is another name of domestic rabbit. There are many breeds in domestic rabbit and the breeds being called Japanese white and New Zealand white are advantageously used.

[0024]

As to basic extracting steps for the present extract, the following steps are used for example.

(A) Inflamed skin tissues of rabbits by the intradermal inoculation with vaccinia virus are collected, and the inflamed tissues are crushed. To the crushed tissues an extraction solvent such as water, phenol water, physiological saline or phenol-added glycerin water is added to conduct an extracting treatment for several days. Then, the mixture is filtrated or centrifuged to give a crude extract (filtrate or supernatant) wherefrom tissue fragments are removed.

(B) The crude extract obtained in (A) is adjusted to acidic pH, heated and then filtered or centrifuged to conduct a deproteinizing treatment. After that, the deproteinized solution is adjusted to basic pH, heated and

then filtered or centrifuged to give a deproteinized filtrate or supernatant.

(C) The filtrate or the supernatant obtained in (B) is adjusted to acidic pH and adsorbed with an adsorbent such as activated carbon or kaolin.

(D) An extraction solvent such as water is added to the adsorbent obtained in (C), the mixture is adjusted to basic pH and the adsorbed component is eluted to give an extract from inflamed skins of rabbits inoculated with vaccinia virus (the present extract).

The above is the basic steps and each of the steps will be more specifically illustrated as follows.

[0025]

About (A):

The inflamed skin tissues of rabbits by the intradermal inoculation of vaccinia virus are collected. The collected skin tissues are washed and disinfected using a phenol solution, etc. This inflamed skin tissues are crushed and an extraction solvent in 1- to 5-fold thereof by volume is added thereto. Here, the term "crush" means to finely break down into minces using a mincing machine or the like. As to the extraction solvent, there may be used distilled water, physiological saline, weakly acidic to weakly basic buffer, etc. and bactericidal/antiseptic agent such as phenol, stabilizer such as glycerin, salts such as sodium chloride, potassium chloride or magnesium chloride, etc. may be appropriately added thereto. At that time, it is also possible that the cell tissue is destroyed by a treatment such as freezing/melting, ultrasonic wave, cell membrane dissolving enzyme or surfactant so as to make the extraction easier. The resulting suspension is allowed to stand for 5 to 12 days. During that period, the suspension may be heated at 30 to 45°C with or without appropriate stirring. The resulting liquid is subjected to a treatment for separating into solid and liquid (filtered or centrifuged, etc.) to remove the tissue fragments whereupon a crude

extract (filtrate or supernatant) is obtained.

[0026]

About (B) The crude extract obtained in (A) is subjected to a deproteinizing treatment. The deproteinization may be carried out by a known method which has been usually conducted and a method such as heating treatment, treatment with a protein denaturant (such as acid, base, urea, guanidine or an organic solvent including acetone), isoelectric precipitation or salting-out may be applied. After that, a common method for the removal of insoluble matters such as filtration using filter paper (such as cellulose or nitrocellulose), glass filter, Celite or Seitz filter, ultrafiltration or centrifugation is conducted to give a filtrate or a supernatant wherefrom the separated insoluble protein is removed.

[0027]

About (C)

The filtrate or supernatant obtained in (B) is adjusted to acidic or, preferably, to pH 3.5 to 5.5 to conduct an operation of adsorbing with an adsorbent. Examples of the usable adsorbent include activated carbon and kaolin. An adsorbent is added to the extract followed by stirring or the extract is passed through a column filled with an adsorbent so that the active ingredient can be adsorbed with the adsorbent. When an adsorbent is added to the extract, the adsorbent with which the active ingredient is adsorbed can be obtained by means of filtration, centrifugation, etc. to remove the solution.

[0028]

About (D)

For elution (desorption) of the active ingredient from the adsorbent obtained in (C), an elution solvent is added to said adsorbent and adjusted to basic or, preferably, to pH 9 to 12, elution is conducted at room temperature or with suitable heating, or with stirring, and then the adsorbent is removed

by a common method such as filtration or centrifugation. As to the extraction solvent used therefore, there may be used a basic solvent such as water, methanol, ethanol, isopropanol or the like adjusted to basic pH or an appropriate mixed solvent thereof and preferably, water adjusted to pH 9 to 12 may be used. Amount of the extracting solvent may be appropriately set. In order to use the eluate obtained as such as a drug substance, the pH is appropriately adjusted to nearly neutral or the like whereby an extract from inflamed skins of rabbits inoculated with vaccinia virus (the present extract) can be finally obtained.

[0029]

Since the present extract is liquid at the stage of being prepared, it is also possible that said extract is appropriately concentrated or diluted to make into a desired concentration. When a preparation is manufactured from the present extract, it is preferred to apply a sterilizing treatment with heating. For making into an injection preparation, it is possible to add sodium chloride or the like so as to prepare a solution being isotonic to physiological saline. It is also possible that the present extract in a liquid state is subjected to an appropriate operation such as concentration to dryness whereby a solid preparation for oral administration such as tablet is manufactured. Specific methods for the manufacture of solid preparation for oral administration from the present extract are disclosed in the specifications of Japanese Patent Nos. 3,818,657 and 4,883,798. The present preparation includes an injection preparation, a solid preparation for oral administration, etc. prepared as such.

[0030]

The content of N-acetylneuraminic acid in the present extract and the present preparation can be measured by the usual quantifying method. To be more specific, there may be used, for example, a measuring method using liquid chromatograph mass spectrometer (LC-MS) or capillary

electrophoretic mass spectrometer (CE-MS), a fluorescence labeling method using DMB (1,2-diamino-4,5-methylenedioxybenzene) and an enzymatic method. In any of those methods, N-acetylneuraminic acid in the present extract and the present preparation can be quantified using a calibration curve prepared by an N-acetylneuraminic acid specimen for calibration.

[0031]

The content of N-acetylneuraminic acid contained in the present extract and the present preparation manufactured by the applicant was measured by the above methods. As a result, although there were some scatters, the present extract and the present preparation contained 4000 ng or more of N-acetylneuraminic acid per unit. To be more specific, the present extract and the present preparation contained 4000 to 18000 ng of N-acetylneuraminic acid per unit. Accordingly, when quality of the present extract and the present preparation is controlled using the content of N-acetylneuraminic acid in the present extract and the present preparation as an index, it was judged to be possible to adopt the content as such as a standard. In the meanwhile, when variations happened in the method for the manufacture of the present extract and the present preparation, the applicant confirmed that the content of N-acetylneuraminic acid per unit may be lower than 4000 ng.

[0032] As will be apparent from the above-mentioned explanation, the term "per unit" used herein means per the content of the active ingredient in the present extract and the present preparation. The present extract (NTP extract) manufactured by the applicant contains 1.2 units/mL of the active ingredient. The present preparation for injection (NTP injection) manufactured using the same also contains 1.2 unit/mL of the active ingredient. In NTP injection, the injection where the volume is 3 mL and 1 mL are available. Accordingly, a preparation of 3 mL contains 3.6 units of the active ingredient and a preparation of 1 mL contains 1.2 units of

the active ingredient. On the other hand, the present preparation for oral administration (NTP tablet) manufactured by the applicant contains 4 units of the active ingredient per tablet.

[0033]

Incidentally, the present extract may be concentrated or diluted. Further, the present preparation containing various units may be also manufactured. In such cases, the amount of the active ingredient contained in a unit amount (such as per mL, per mg, per ampoule, per tablet, etc.) of the present extract and the present preparation may also vary. Therefore, it is basically meaningful that the content of N-acetylneuraminic acid is stipulated in relation to the amount of the active ingredient of the present extract and the present preparation. That is because such a thing is connected to the relation to efficacy and safety of the present extract or the present preparation. Accordingly, the applicant stipulated the content of N-acetylneuraminic acid in the present extract and the present preparation in terms of "per the content of the active ingredient" ("per unit"). On the other hand, since the applicant actually manufactures and distributes NTP preparation, it is also certainly meaningful to stipulate per mL or per ampoule of the specific injection or to stipulate per tablet in relation to the dose whereby such stipulation was also adopted.

[0034]

In the meanwhile, in NTP injection, there are generic drugs or similar drugs (hereinafter, they will be referred to as "other company's injections") manufactured by the companies other than the applicant's company (hereinafter, they will be referred to as "other companies") in Japan and in China. For other company's injections, an extract from inflamed skins of rabbits inoculated with vaccinia virus (the present extract) which is an active ingredient is also quantitatively determined by using its analgesic effect as an index using SART stress mice. In terms of the indication, the

content of the active ingredient in other company's injections is merely mentioned as "unit" in plural other company's injections in Japan which are "ROSEMORGGEN Inj." (registered trade mark), "NABUTOPIN Inj." (registered trade mark) and "NOLPORT Inj." (registered trade mark), or is mentioned as "Analgecine unit" or "AGC unit" in one other company's injection in China which is "恩再適/ANALGECINE" (registered trade mark). However, the same as NTP injection, in any of said preparations, the content of the active ingredient is 1.2 units or 1.2 Analgecine (1.2 AGC) units per mL or 3.6 units or 3.6 Analgecine (3.6 AGC) units per ample of 3 mL preparation. To sum up, the "Neurotropin unit" used by NTP preparation and the "unit" or "Analgecine unit" used by other companies are the same measure for stipulating the content of the active ingredient, and they are different just in terms of the expression. Under such circumstances, "unit" is used in the present application in expressing the content of the present extract which is an active ingredient not only for NTP preparation but also for all other company's injections. As such, where the amount of an extract from inflamed skins of rabbits inoculated with vaccinia virus which is an active ingredient is expressed by using "unit" in plural preparations. Therefore, with regard to said preparations, the expression in terms of "unit" is clear for persons skilled in the art.

[0035]

As will be apparent from the above, "the present preparation" in the present application is a conception including NTP preparation (NTP injection and NTP tablet) and other company's injections. When other companies will manufacture and distribute tablets (hereinafter, it will be referred to as "other company's tablets") as generic drug or similar drug for NTP tablet in future, the above conception also covers other company's tablets as such.

[0036]

As hereunder, specific examples for the manufacture of the present extract and the present preparation and for the result of measurement of the content of N-acetylneuraminic acid in the present extract and the present preparation are shown as Examples although the present invention is not limited at all by the description of those Examples.

#### [Examples]

##### [0037]

###### Example 1 (Manufacture of the present extract)

Skins of healthy adult rabbits were inoculated with vaccinia virus intradermally and the inflamed skins were cut and collected. The collected skins were washed and disinfected by a phenol solution, an excessive phenol solution was removed and the residue was crushed. A phenol solution was added thereto and mixed therewith and the mixture was allowed to stand for 3 to 7 days, and further heated at 35 to 40°C together with stirring for 3 to 4 days. After that, an extracted solution obtained by a solid-liquid separation was adjusted to pH 4.5 to 5.2 with hydrochloric acid, heated at 90 to 100°C for 30 minutes and filtered to remove protein. The filtrate was adjusted to pH 9.0 to 9.5 with sodium hydroxide, heated at 90 to 100°C for 15 minutes and subjected to a solid-liquid separation.

##### [0038]

The resulting deproteinized solution was adjusted to pH 4.0 to 4.3 with hydrochloric acid, activated carbon in an amount of 2% to the mass of the deproteinized solution was added thereto and the mixture was stirred for 2 hours and subjected to the solid-liquid separation. Water was added to the collected activated carbon followed by adjusting to pH 9.5 to 10 with sodium hydroxide and the mixture was stirred at 60°C for 90 to 100 minutes and centrifuged to give a supernatant. Water was added again to the activated carbon precipitated upon the centrifugation followed by adjusting

to pH 10.5 to 11 with sodium hydroxide and the mixture was stirred at 60°C for 90 to 100 minutes and centrifuged to give a supernatant. Both supernatants were combined and neutralized with hydrochloric acid to give the present extract.

[0039]

Example 2 (Method for measuring the content of N-acetylneuraminic acid)

The content of N-acetylneuraminic acid in the present extract and the present preparation was measured as follows using a high-performance liquid chromatographic mass spectrometer (LC-MS).

The present extract (1.2 units/mL) manufactured according to Example 1 was diluted with water to an extent of 10-fold and injected into the LC-MS.

The present preparation (NTP injection) manufactured using the present extract which was manufactured according to Example 1 was also diluted with water to an extent of 10-fold and injected into the LC-MS.

With regard to the present preparation (NTP tablet) manufactured using the present extract which was manufactured according to Example 1, three tablets thereof were washed with 3 mL of methanol/chloroform (1:1) for three times to remove the film coat layer, dried, suspended in 12 mL of water (1 unit/mL). After centrifugation of the suspension, the supernatant was diluted with water to an extent of 10-fold and injected into the LC-MS.

With regard to N-acetylneuraminic acid, a standard solution in an aqueous solution was prepared and a calibration curve was produced.

[0040]

In the LC-MS, the 1100 Series manufactured by Agilent Technologies, Inc. and API 3000 (manufactured by Applied Biosystems/MDS Sciex) were used for an HPLC part and a mass spectrometer, respectively. Analytical conditions are as follows.

Column: Inertsil ODS-3 ( $\phi$  2.1 × 150 mm)  
Column temperature: 25°C; flow rate: 200  $\mu$ L/minute  
Mobile phase: methanol/0.05% formic acid  
Methanol %/minute: 0/0-0/1-44/8-100/8.1-100/11  
Injection amount: 5  $\mu$ L; temperature set for the sample chamber: 4°C  
Detection: positive ion detecting MRM  
Measuring conditions for the LC-MS are shown in Table 1. Each parameter in Table 1 means as follows.

- DP: voltage applied to orifice plate
- FP: voltage applied to focus ring
- CE: collision energy
- CXP: voltage applied to the outlet of Q2
- NEB: pressure of nebulizer gas
- CUR: pressure of curtain gas
- IS: voltage of ion spray
- CAD: pressure of collision gas
- TEM: turbo gas temperature

[0041]

[Table 1]

Detected Ion (Q1/Q3)	DP	FP	CE	CXP	NEB	CUR	IS	CAD	TEM
310/274	20	100	20	20	12	10	5000	8	450

[0042]

Example 3 (Measured result of the content of N-acetylneuraminic acid in the present extract)

Result of measurement of the content of N-acetylneuraminic acid in the present extract by the method mentioned in Example 2 is shown in Table

2. Content of the active ingredient in the present extract is 1.2 units/mL. The content of N-acetylneuraminic acid in the present extract is expressed in both terms of per unit of the present extract (“/unit”) and per mL of the present extract (“/mL”). Incidentally, the references A to C in lot numbers show the difference in the manufacturing places (institutions) of the applicant. Measured values were adjusted to three significant figures (Hereinafter, the above is the same for all of measured values.).

[0043]

[Table 2]

Lot No.	Content of N-acetylneuraminic acid	
	(ng/unit)	(ng/mL)
A01	9920	11900
A02	9670	11600
A03	5980	7180
B01	7490	8990
B02	7360	8830
B03	8420	10100
B04	6830	8200
B05	7690	9230
B06	10100	12100
B07	8420	10100
B08	8830	10600
B09	12000	14400
B10	11700	14000
C01	8060	9670
C02	9250	11100
C03	9580	11500
C04	8920	10700
C05	9250	11100
C06	10800	13000
C07	9750	11700
C08	10500	12600
C09	9170	11000
C10	6290	7550

C11	7800	9360
C12	7490	8990
C13	11800	14200
C14	11400	13700
C15	10200	12200
C16	6750	8100
C17	8750	10500
C18	9500	11400
C19	7540	9050
C20	9670	11600
C21	9750	11700
C22	13400	16100
C23	6800	8160
C24	7420	8900
C25	8670	10400
Mean	9020	10800

[0044]

Example 4 (Measured result 1 of the content of N-acetylneuraminic acid in the present preparation)

The result of measurement of the content of N-acetylneuraminic acid in NTP injection (containing 1.2 units per mL) among the present preparation by the method mentioned in Example 2 is shown in Table 3. The result is shown in terms of the content per unit of the active ingredient of the present preparation ("/unit"), per mL of the present preparation ("/mL") and per ampoule containing 3 mL ("/ampoule").

[0045]

[Table 3]

Type of the present preparation (Identification No.)	Content of N-acetylneuraminic acid		
	(ng/unit)	(ng/mL)	(ng/ampoule)
NTP injection (NI01)	7450	8940	26800
NTP injection (NI02)	8420	10100	30300
NTP injection (NI03)	6540	7850	23600

NTP injection (NI04)	9500	11400	34200
NTP injection (NI05)	9580	11500	34500
NTP injection (NI06)	10400	12500	37500
Mean	8650	10400	31200

[0046]

Example 5 (Measured result 2 of the content of N-acetylneuraminic acid in the present preparation)

The result of measurement of the content of N-acetylneuraminic acid in NTP tablet (containing 4 units per tablet) among the present preparation by the method mentioned in Example 2 is shown in Table 4. The result is shown in terms of the content per unit of the active ingredient ("/unit") and per tablet ("/tablet").

[0047]

[Table 4]

Type of the present preparation (Identification No.)	Content of N-acetylneuraminic acid	
	(ng/unit)	(ng/tablet)
NTP tablet (NT01)	12600	50400
NTP tablet (NT02)	4780	19100
NTP tablet (NT03)	10500	42000
NTP tablet (NT04)	8700	34800
NTP tablet (NT05)	10200	40800
NTP tablet (NT06)	9840	39400
NTP tablet (NT07)	9650	38600
NTP tablet (NT08)	8950	35800
NTP tablet (NT09)	9490	38000
NTP tablet (NT10)	7740	31000
NTP tablet (NT11)	8090	32400

NTP tablet (NT12)	9380	37500
NTP tablet (NT13)	6990	28000
Mean	8990	36000

[0048]

Comparative Example 1 (Measured result of the content of N-acetylneuraminic acid in other company's injections)

The result of measurement of the content of N-acetylneuraminic acid in other company's injections by the same manner as in NTP preparation is shown in Table 5. Incidentally, the distribution of "ROSEMORGEN Inj." has been already finished at the stage of filing the present application and is not available in the market and the following measured result is a result of measurement for the thing which was formerly purchased by the applicant in the market.

[0049]

[Table 5]

Type of other company's injections (Lot No.)	Content of N-acetylneuraminic acid	
	(ng/unit)	(ng/mL)
ROSEMORGEN Inj. (2E27B)	141	169
ROSEMORGEN Inj. (4E57A)	142	170
NOLPORT Inj. (678405)	72.8	87.4
NOLPORT Inj. (709203)	305	366
NABUTOPIN Inj. (BD597E)	54.2	65.0
NABUTOPIN Inj. (KF606E)	170	204
ANALGECINE (20071209)	378	454
ANALGECINE (20080301)	377	452

[0050]

From the above results, it is noted that, even in the same preparation of the extract from inflamed skins of rabbits inoculated with vaccinia virus, the content of N-acetylneuraminic acid is greatly different depending upon the manufacturing company (Tables 3 to 5). It is further noted that, even in the preparation of the same company including the applicant, the content of N-acetylneuraminic acid is sometimes considerably different (Tables 3 to 5). For an extract from inflamed skins of rabbits inoculated with vaccinia virus which is a drug substance for the preparation of the extract from inflamed skins of rabbits inoculated with vaccinia virus, those drug substances of other companies are not available whereby only that which was manufactured by the applicant (NTP extract) was measured. It is noted that, for such a one, there are also scatters to some extent in the content of N-acetylneuraminic acid (Table 2).

[0051]

However, in the present extract and the present preparation manufactured by the applicant, N-acetylneuraminic acid was contained in an amount of not less than 4000 ng/unit (Tables 2 to 4). Further, in the present extract and the present preparation manufactured by the applicant, N-acetylneuraminic acid was contained within a range of 4000 to 22500 ng/unit. On the contrary, among the present preparation manufactured by other companies, none of them contained such a high amount of N-acetylneuraminic acid (Table 5). Although it is not clear what is the cause for the difference in the contents of N-acetylneuraminic acid in the present preparation manufactured by each of the companies, it is strongly presumed to be caused by the difference in the manufacturing method for the present preparation in each company. Anyway, as mentioned already, N-acetylneuraminic acid has an analgesic action or, particularly, an effect for non-inflammatory pain diseases such as neuropathic pain disease whereby the applicant understands that the fact itself that this substance

(component) is contained in more amount in the present preparation (NTP injection) manufactured by the applicant as compared with the injections by other companies constitutes the characteristic and is a favorable characteristic.

[0052]

As mentioned hereinabove, it is the characteristic of the present extract and the present preparation that not less than 4000 ng/unit of N-acetylneuraminic acid is contained in the present extract and the present preparation. Similarly, it is the characteristic of the present extract and the present preparation that, when the measurement is done by the measuring method of the content of N-acetylneuraminic acid in the present extract and the present preparation (injection and tablet) mentioned in Example 2 (hereinafter, it will be referred to as "10-fold dilution method"), N-acetylneuraminic acid is contained within a range of 4000 to 15000 ng/unit in the present extract and the present preparation. It constitutes the characteristic of the present extract and the present preparation that N-acetylneuraminic acid is contained within a range of 4000 to 22500 ng/unit when the content of N-acetylneuraminic acid in the present extract and the present preparation is measured by a measuring method using other dilution ratio such as 50-fold dilution or 250-fold dilution with water (hereinafter, it will be referred to as a "different dilution method") instead of 10-fold dilution method where the dilution ratio with water is 10-fold. The reason why the upper limit hereinabove is 1.5 times more as compared with the case of 10-fold dilution method will be mentioned later.

[0053]

Further, with regard to the injection containing 1.2 units per mL (hereinafter, it will be referred to as "the present injection") among the present preparation, it constitutes the characteristic of the present injection that not less than 4800 ng/mL of N-acetylneuraminic acid is contained

therein. Similarly, it constitutes the characteristic of the present injection that, when measurement is done by the 10-fold dilution method, N-acetylneuraminic acid is contained within a range of 4800 to 18000 ng/mL. It constitutes the characteristic of the present injection that, when the content of N-acetylneuraminic acid in the present injection is measured by a different dilution method, N-acetylneuraminic acid is contained within a range of 4800 to 27000 ng/mL. Further, when the present injection is that filled with 3 mL volume in an ampoule (hereinafter, the present injection as such will be referred to as "the present injection of 3 mL ampoule"), it constitutes the characteristic of the present injection of 3 mL ampoule that N-acetylneuraminic acid is contained in an amount of not less than 14400 ng per ampoule. Similarly, it constitutes the characteristic of the present injection of 3 mL ampoule that, when measurement is done by a 10-fold dilution method, N-acetylneuraminic acid is contained within the range of 14400 to 54000 ng/ampoule. It constitutes the characteristic of the present injection of 3 mL ampoule that, when the content of N-acetylneuraminic acid in the present injection of 3 mL ampoule is measured by a different dilution method, N-acetylneuraminic acid is contained within the range of 14400 to 81000 ng/ampoule. The reason why the upper limit hereinabove is 1.5 times more as compared with the case of the 10-fold dilution method will be also mentioned later.

[0054]

Further, with regard to the tablet containing 4 units per tablet (hereinafter, it will be referred to as "the present tablet") among the present preparation, it constitutes the characteristic of the present tablet that not less than 16000 ng/tablet of N-acetylneuraminic acid is contained therein. Similarly, it constitutes the characteristic of the present tablet that, when measurement is done by the 10-fold dilution method, N-acetylneuraminic acid is contained within the range of 16000 to 60000 ng/tablet. It

constitutes the characteristic of the present tablet that, when the content of N-acetylneuraminic acid in the present tablet is measured by a different dilution method, N-acetylneuraminic acid is contained within the range of 16000 to 90000 ng/tablet. The reason why the upper limit hereinabove is 1.5 times more as compared with the case of the 10-fold dilution method will be also mentioned later.

[0055]

In view of the above, it is possible that the content of N-acetylneuraminic acid contained in the present extract and the present preparation is measured so as to confirm whether the present extract and the present preparation are appropriately manufactured whereby the quality thereof is controlled. Thus, it is possible that, when the content of N-acetylneuraminic acid contained in the present extract and the present preparation is measured and, if it is found to be 4000 ng/unit or more, that is evaluated as being appropriately manufactured. It is further possible to conclude that the present extract and the present preparation are appropriately manufactured by means of such a confirmation that, when the content of N-acetylneuraminic acid contained in the present extract and the present preparation is measured by the 10-fold dilution method or by different dilution methods and is found to be within the range of 4000 to 15000 ng/unit or 4000 to 22500 ng/unit, respectively.

[0056]

In the case of the present injection, it can be judged that the present injection is appropriately manufactured when the content of N-acetylneuraminic acid contained in the present injection is measured and is found to be 4800 ng/mL or more. It is further possible to judge that the present injection is appropriately manufactured by measuring the content of N-acetylneuraminic acid contained in the present injection by a 10-fold dilution method or by a different dilution method and by confirming to be

within the range of 4800 to 18000 ng/unit or 4800 to 27000 ng/unit, respectively. Similarly, in the case of the present injection of 3 mL ampoule, it is also possible to judge that the 3 mL ampoule injection is appropriately manufactured when the content of N-acetylneuraminic acid contained therein is measured and is found to be 14400 ng/mL or more or when it is within the range of 14400 to 54000 ng/unit or within the range of 14400 to 81000 ng/mL in the case of measurement by a 10-fold dilution method or by a different dilution method, respectively.

[0057]

In the case of the present tablet, it can be evaluated that, when the content of N-acetylneuraminic acid contained in the present tablet is measured and is found to be 16000 ng/tablet or more, the said tablet is judged to be appropriately manufactured. It is further possible that, when the content of N-acetylneuraminic acid contained in the present tablet is measured by a 10-fold dilution method or by a different dilution method and is confirmed to be within the range of 16000 to 60000 ng/unit or 16000 to 90000 ng/tablet, respectively, then the said present tablet is judged to be appropriately manufactured.

[0058]

Incidentally, the fact that the value corresponding to the upper limit when the above content of N-acetylneuraminic acid is shown in terms of the range of from lower limit to upper limit as measured by a different dilution method is considerably high (about 1.5 times as much) as compared with the measured result (Tables 2 to 4) obtained in Examples 3 to 5 is due to the following reason and grounds.

[0059]

Thus, in the above Examples, the content of N-acetylneuraminic acid is measured by means of LC-MS, and N-acetylneuraminic acid is apt to be affected by impurity ions (ion suppression). Basically, the present inventors

measure a sample of the present extract, etc. after diluting to an extent of 10-fold but, when diluted to an extent of 50-fold or 250-fold, higher values were sometimes noted than the measured values in the case of the 10-fold dilution. It is likely that such a phenomenon is due to the fact that, in case the dilution degree is high, the affection by the concentration of impurity ions lowers. As a result of inspection by the present inventors comparing the quantified values in various dilution degrees in plural samples, it was confirmed that, upon dilution to an extent of 50-fold or 250-fold, the value of about 1.5 times in maximum as compared with the case of 10-fold dilution was observed (Table 6). Based upon such a fact, the value corresponding to the upper limit of the content of N-acetylneuraminic acid was set as mentioned above in the present application.

[0060]

[Table 6]

Identification No. or Lot No.	Content of N-acetylneuraminic acid		
	(ng/unit) 10-fold dilution sample	(ng/unit) 50-fold dilution sample	(ng/unit) 250-fold dilution sample
A01	9920	10400	9830
A01	9670	10700	9750
A02	5980	6210	6460
B09	12000	13300	12700
B10	11700	13200	13900
C24	7420	8030	8100
C25	8670	10200	9750
NTP injection (NI04)	9500	12200	11300
NTP injection (NI05)	9580	12500	11400
NTP injection (NI06)	10400	13500	13800
NTP tablet (NT03)	10500	14100	12000

NTP tablet (NT04)	8700	12300	11200
NTP tablet (NT05)	10200	13900	13000
NTP tablet (NT06)	9840	13200	12300
NTP tablet (NT07)	9650	12300	11600
NTP tablet (NT08)	8950	11400	10700
NTP tablet (NT09)	9490	12000	11900
NTP tablet (NT10)	7740	9640	10000
NTP tablet (NT11)	8090	11300	10400
NTP tablet (NT12)	9380	11900	11800
NTP tablet (NT13)	6990	8990	8700

[0061]

In view of the above, the following inventions can be induced as the present invention although those are mere exemplification and the present invention is not limited thereto. Incidentally, in each of the inventions as shown below, the passage reading "by measuring the amount of N-acetylneuraminic acid" is usually used in such a meaning that the amount of N-acetylneuraminic acid is measured for each manufacturing lot of the present extract or the present preparation.

(1) In an extract from inflamed skins of rabbits inoculated with vaccinia virus, the extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4000 ng or more per unit of the extract.

(2) In an extract from inflamed skins of rabbits inoculated with vaccinia virus, the extract from inflamed skins of rabbits inoculated with

vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4000 to 22500 ng per unit of the extract.

(3) In an extract which is liquid from inflamed skins of rabbits inoculated with vaccinia virus, the extract being liquid from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4800 ng or more per mL of the extract.

(4) In an extract which is liquid from inflamed skins of rabbits inoculated with vaccinia virus, the extract being liquid from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4800 to 27000 ng per mL of the extract.

(5) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(6) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 ng to 22500 ng per unit of the extract from

inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(7) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped by being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(8) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped by being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(9) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped by complying with the standard that the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is found to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(10) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped by complying with the standard that the amount of N-acetylneuraminic acid contained in the preparation is measured and the

content is found to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(11) In an injection preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injection preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injection preparation is measured and the content is confirmed to be 4800 ng or more per mL of the injection preparation.

(12) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injection preparation is measured and the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation.

(13) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 4800 ng or more per mL of the injectable preparation.

(14) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 4800 to

27000 ng per mL of the injectable preparation.

(15) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is 4800 ng or more per mL of the injectable preparation.

(16) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is 4800 to 27000 ng per mL of the injectable preparation.

(17) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(18) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(19) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable

preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(20) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(21) In a 3-mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3-mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(22) In a 3-mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3-mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard by such means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(23) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be

appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 ng or more per tablet.

(24) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 to 90000 ng per tablet.

(25) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 ng or more per tablet.

(26) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after being judged to be appropriately manufactured by such means that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 to 90000 ng per tablet.

(27) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is 16000 ng or more per tablet.

(28) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from

inflamed skins of rabbits inoculated with vaccinia virus which is shipped after complying with the standard that the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is 16000 to 90000 ng per tablet.

(29) The preparation according to any of (5) to (10), wherein it is an analgesic agent.

(30) The injectable preparation according to any of (11) to (22), wherein it is an analgesic agent.

(31) The tablet according to any of (23) to (28), wherein it is an analgesic agent.

[0062]

(32) In an extract from inflamed skins of rabbits inoculated with vaccinia virus, the extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4000 ng or more per unit of the extract.

(33) In an extract from inflamed skins of rabbits inoculated with vaccinia virus, the extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4000 to 22500 ng per unit of the extract.

(34) In an extract which is liquid from inflamed skins of rabbits inoculated with vaccinia virus, the extract being liquid from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4800 ng or more per mL of the extract.

(35) In an extract being liquid from inflamed skins of rabbits inoculated with vaccinia virus, the extract being liquid from inflamed skins of rabbits inoculated with vaccinia virus where the amount of

N-acetylneuraminic acid contained in the extract is measured and the content is confirmed to be 4800 to 27000 ng per mL of the extract.

(36) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccine virus in the preparation.

(37) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the preparation is measured and the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccine virus in the preparation.

(38) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 4800 ng or more per mL of the injectable preparation.

(39) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation.

(40) In a 3 mL injectable preparation containing an extract from

inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(41) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation is measured and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(42) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 ng or more per tablet.

(43) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the tablet is measured and the content is confirmed to be 16000 to 90000 ng per tablet.

(44) The preparation according to (36) or (37), wherein it is an analgesic agent.

(45) The injectable preparation according to any of (38) to (41), wherein it is an analgesic agent.

(46) The tablet according to (42) or (43), wherein it is an analgesic agent.

[0063]

(47) In a preparation containing an extract from inflamed skins of

rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the preparation before shipping is measured and the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccine virus in the preparation.

(48) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the preparation before shipping is measured and the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccine virus in the preparation.

(49) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation before shipping is measured and the content is confirmed to be 4800 ng or more per mL of the injectable preparation.

(50) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation before shipping is measured and the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation.

(51) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained

in the injectable preparation before shipping is measured and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(52) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the injectable preparation before shipping is measured and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(53) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the tablet before shipping is measured and the content is confirmed to be 16000 ng or more per tablet.

(54) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the tablet before shipping is measured and the content is confirmed to be 16000 to 90000 ng per tablet.

(55) The preparation according to (47) or (48), wherein it is an analgesic agent.

(56) The injectable preparation according to any of (49) to (52), wherein it is an analgesic agent.

(57) The tablet according to (53) or (54), wherein it is an analgesic agent.

[0064]

(58) A method for inspection where the amount of N-acetylneuraminic acid contained in an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 4000 ng or more per unit of the extract, manufacture of the extract is judged

to be appropriately conducted.

(59) A method for inspection where the amount of N-acetylneuraminic acid contained in an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 4000 to 22500 ng per unit of the extract, manufacture of the extract is judged to be appropriately conducted.

(60) A method for inspection where the amount of N-acetylneuraminic acid contained in a preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation, manufacture of the extract is judged to be appropriately conducted.

(61) A method for inspection where the amount of N-acetylneuraminic acid contained in a preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation, manufacture of the extract is judged to be appropriately conducted.

(62) A method for inspection where the amount of N-acetylneuraminic acid contained in an injectable preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 4800 ng or more per mL of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(63) A method for inspection where the amount of N-acetylneuraminic acid contained in an injectable preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia

virus is measured and, when the content is 4800 to 27000 ng per mL of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(64) A method for inspection where the amount of N-acetylneuraminic acid contained in a 3 mL injectable preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 14400 ng or more per ampoule of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(65) A method for inspection where the amount of N-acetylneuraminic acid contained in a 3-mL injectable preparation which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 14400 to 81000 ng per ampoule of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(66) A method for inspection where the amount of N-acetylneuraminic acid contained in a tablet which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 16000 ng or more per tablet, manufacture of the tablet is judged to be appropriately conducted.

(67) A method for inspection where the amount of N-acetylneuraminic acid contained in a tablet which contains an extract from inflamed skins of rabbits inoculated with vaccinia virus is measured and, when the content is 16000 to 90000 ng per tablet, manufacture of the tablet is judged to be appropriately conducted.

[0065]

(68) A method for controlling the manufacture of an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the extract is 4000 ng or

more per unit of the extract, manufacture of the extract is judged to be appropriately conducted.

(69) A method for controlling the manufacture of an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the extract is 4000 to 22500 ng per unit of the extract, manufacture of the extract is judged to be appropriately conducted.

(70) A method for controlling the manufacture of a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the preparation is 4000 ng or more per unit of the extract in the preparation, manufacture of the preparation is judged to be appropriately conducted.

(71) A method for controlling the manufacture of a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the preparation is 4000 to 22500 ng per unit of the extract in the preparation, manufacture of the preparation is judged to be appropriately conducted.

(72) A method for controlling the manufacture of an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the injectable preparation is 4000 ng or more per mL of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(73) A method for controlling the manufacture of an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the injectable preparation is 4800 to 27000 ng per mL of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(74) A method for controlling the manufacture of a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the 3 mL injectable preparation is 14400 ng or more per ampoule of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(75) A method for controlling the manufacture of a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the 3 mL injectable preparation is 14400 to 81000 ng per ampoule of the injectable preparation, manufacture of the injectable preparation is judged to be appropriately conducted.

(76) A method for controlling the manufacture of a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the tablet is 16000 ng or more per tablet, manufacture of the tablet is judged to be appropriately conducted.

(77) A method for controlling the manufacture of a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where, when the amount of N-acetylneuraminic acid contained in the tablet is 16000 to 90000 ng per tablet, manufacture of the tablet is judged to be appropriately conducted.

[0066]

(78) Use of an extract from inflamed skins of rabbits inoculated with vaccinia virus where the content of N-acetylneuraminic acid in the manufacture of a preparation containing the extract is 4000 ng or more per unit of the extract.

(79) Use of an extract from inflamed skins of rabbits inoculated with vaccinia virus where the content of N-acetylneuraminic acid in the

manufacture of a preparation containing the extract is 4000 to 22500 ng per unit of the extract.

(80) Use of an extract from inflamed skins of rabbits inoculated with vaccinia virus where the content of N-acetylneuraminic acid in the manufacture of a preparation containing the extract is 4800 ng or more per mL of the extract.

(81) Use of an extract from inflamed skins of rabbits inoculated with vaccinia virus where the content of N-acetylneuraminic acid in the manufacture of a preparation containing the extract is 4800 to 27000 ng per mL of the extract.

[0067]

(82) A method for shipping a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the preparation is measured and, when the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(83) A method for shipping a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the preparation is measured and, when the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(84) A method for shipping an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the injectable preparation is measured and, when the content is confirmed to be 4800 ng or more per mL

of the injectable preparation, the injectable preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(85) A method for shipping an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the injectable preparation is measured and, when the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation, the injectable preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(86) A method for shipping a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the injectable preparation is measured and, when the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation, the 3 mL injectable preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(87) A method for shipping a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where amount of N-acetylneuraminic acid contained in the injectable preparation is measured and, when the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation, the 3 mL injectable preparation is judged to be appropriately manufactured and is judged to be able to be shipped.

(88) A method for shipping a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of N-acetylneuraminic acid contained in the tablet is measured and, when the content is confirmed to be 16000 ng or more per tablet, the tablet is judged to be appropriately manufactured and is judged to be able to be shipped.

(89) A method for shipping a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus where the amount of

N-acetylneuraminic acid contained in the tablet is measured and, when the content is confirmed to be 16000 to 90000 ng per tablet, the tablet is judged to be appropriately manufactured and is judged to be able to be shipped.

(90) A preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped according to the shipping method mentioned in (82) or (83).

(91) An injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped according to the shipping method mentioned in any of (84) to (87).

(92) A tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is shipped according to the shipping method mentioned in (88) or (89).

[0068]

(93) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation where the variations in the effect of the preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(94) In a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation where the variations in the effect of the preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(95) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation

where the variations in the effect of the injectable preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and the content is confirmed to be 4800 ng or more per mL of the injectable preparation.

(96) In an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the injectable preparation where the variations in the effect of the injectable preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation.

(97) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation where the variations in the effect of the injectable preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation.

(98) In a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the 3 mL injectable preparation where the variations in the effect of the injectable preparation among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation.

(99) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet where the variations in the effect of the tablet among the manufacturing lots are reduced by such a means that

the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and the content is confirmed to be 16000 ng or more per tablet.

(100) In a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the tablet where the variations in the effect of the tablet among the manufacturing lots are reduced by such a means that the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and the content is confirmed to be 16000 to 90000 ng per tablet.

(101) The preparation according to (93) or (94), wherein the effect is an analgesic effect.

(102) The preparation according to (101), wherein the analgesic effect is an analgesic effect for non-inflammatory pain disease.

(103) The injectable preparation according to any of (95) to (98), wherein the effect is an analgesic effect.

(104) The injectable preparation according to (103), wherein the analgesic effect is an analgesic effect for non-inflammatory pain disease.

(105) The tablet according to (99) or (100), wherein the effect is an analgesic effect.

(106) The tablet according to (105), wherein the analgesic effect is an analgesic effect for non-inflammatory pain disease.

[0069]

(107) A method for manufacturing a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and that the content is 4000 ng or more per unit of the

extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(108) A method for manufacturing a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and that the content is 4000 to 22500 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(109) A method for manufacturing an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the injectable preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the injectable preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and that the content is 4800 ng or more per mL of the injectable preparation.

(110) A method for manufacturing an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the injectable preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the injectable preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and that the content is 4800 to 27000 ng per mL of the injectable preparation.

(111) A method for manufacturing a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia

virus in which the variations in the effect of the injectable preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the 3 mL injectable preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and that the content is 14400 ng or more per ampoule of the injection.

(112) A method for manufacturing a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the injectable preparation among manufacturing lots are reduced, wherein it is judged that manufacture of the 3 mL injectable preparation is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and that the content is 14400 to 81000 ng per ampoule of the injection.

(113) A method for manufacturing a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the tablet among manufacturing lots are reduced, wherein it is judged that manufacture of the tablet is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and that the content is 16000 ng or more per tablet.

(114) A method for manufacturing a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which the variations in the effect of the tablet among manufacturing lots are reduced, wherein it is judged that manufacture of the tablet is appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and that the content is 16000 to 90000 ng per tablet.

(115) The method for manufacturing according to any of (107) to (114),

wherein the effect is an analgesic effect.

(116) The method for manufacturing according to (115), wherein the analgesic effect if an analgesic effect for non-inflammatory pain disease.

(117) A preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus manufactured by the method according to (107) or (108).

(118) An injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus manufactured by the method according to any of (109) to (112).

(119) A tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus manufactured by the method according to (113) or (114).

(120) A preparation, an injectable preparation or a tablet manufactured by the method for manufacturing according to (115).

(121) A preparation, an injectable preparation or a tablet manufactured by the method for manufacturing according to (116).

[0070]

(122) A method for inspection of a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the preparation are reduced, wherein manufacture of the preparation is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the preparation is measured for each lot and that the content is 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(123) A method for inspection of a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the preparation are reduced, wherein manufacture of the preparation is judged to be appropriately conducted in such a case that

the amount of N-acetylneuraminic acid contained in the preparation is measured for each lot and that the content is 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus in the preparation.

(124) A method for inspection of an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation are reduced, wherein manufacture of the injectable preparation is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each lot and that the content is 4800 ng or more per mL of the injectable preparation.

(125) A method for inspection of an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation are reduced, wherein manufacture of the injectable preparation is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each lot and that the content is 4800 to 27000 ng per mL of the injectable preparation.

(126) A method for inspection of a 3·mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation are reduced, wherein manufacture of the 3 mL injectable preparation is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each lot and that the content is 14400 ng or more per ampoule of the injectable preparation.

(127) A method for inspection of a 3 mL injectable preparation

containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation are reduced, wherein manufacture of the 3 mL injectable preparation is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each lot and that the content is 14400 to 81000 ng per ampoule of the injectable preparation.

(128) A method for inspection of a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the tablet are reduced, wherein manufacture of the tablet is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the tablet is measured for each lot and that the content is 16000 ng or more per tablet.

(129) A method for inspection of a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the tablet are reduced, wherein manufacture of the tablet is judged to be appropriately conducted in such a case that the amount of N-acetylneuraminic acid contained in the tablet is measured for each lot and that the content is 16000 to 90000 ng per tablet.

(130) The method for inspection according to any of (122) to (129), wherein the effect is an analgesic effect.

(131) The method for inspection according to (130), wherein the analgesic effect of such an analgesic effect for non-inflammatory pain disease.

(132) A preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus inspected by the method of inspection according to (122) or (123).

(133) An injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus inspected by the method of

inspection according to any of (124) to (127).

(134) A tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus inspected by the method of inspection according to (128) or (129).

(135) A preparation, an injectable preparation or a tablet inspected by the method for inspection according to (130).

(136) A preparation, an injectable preparation or a tablet inspected by the method for inspection according to (131).

[0071]

(137) A method for shipping a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and, in case the content is confirmed to be 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation is judged to be appropriately manufactured.

(138) A method for shipping a preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the preparation is measured for each manufacturing lot and, in case the content is confirmed to be 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus, the preparation is judged to be appropriately manufactured.

(139) A method for shipping an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic

acid contained in the injectable preparation is measured for each manufacturing lot and, in case the content is confirmed to be 4800 ng or more per mL of the injectable preparation, the injectable preparation is judged to be appropriately manufactured.

(140) A method for shipping an injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the injectable preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and, in case the content is confirmed to be 4800 to 27000 ng per mL of the injectable preparation, the injectable preparation is judged to be appropriately manufactured.

(141) A method for shipping a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the 3 mL injectable preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and, in case the content is confirmed to be 14400 ng or more per ampoule of the injectable preparation, the injectable preparation is judged to be appropriately manufactured.

(142) A method for shipping a 3 mL injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the 3 mL injectable preparation among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is measured for each manufacturing lot and, in case the content is confirmed to be 14400 to 81000 ng per ampoule of the injectable preparation, the injectable preparation is judged to be appropriately manufactured.

(143) A method for shipping a tablet containing an extract from

inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the tablet among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and, in case the content is confirmed to be 16000 ng or more per tablet, the injectable preparation is judged to be appropriately manufactured.

(144) A method for shipping a tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus in which variations of the effect of the tablet among the manufacturing lots are reduced, wherein the amount of N-acetylneuraminic acid contained in the tablet is measured for each manufacturing lot and, in case the content is confirmed to be 16000 to 90000 ng per tablet, the injectable preparation is judged to be appropriately manufactured.

(145) The method for shipping according to any of (137) to (144), wherein the effect is an analgesic effect.

(146) The method for shipping according to (145), wherein the analgesic effect of such an analgesic effect for non-inflammatory pain disease.

(147) A preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus shipped by the method of shipping according to (137) or (138).

(148) An injectable preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus shipped by the method of shipping according to any of (139) to (142).

(149) A tablet containing an extract from inflamed skins of rabbits inoculated with vaccinia virus shipped by the method of shipping according to (143) or (144).

(150) A preparation, an injectable preparation or a tablet shipped by the method for shipping according to (145).

(151) A preparation, an injectable preparation or a tablet shipped by the method for shipping according to (146).

[0072]

(152) In a method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus, the method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus which is characterized in that an extraction solvent is added to crushed inflamed tissues and allowed to stand for 5 to 12 days.

(153) The method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus according to (152), wherein warming at 30 to 45°C with or without stirring is conducted during the period where an extraction solvent is added to crushed inflamed tissues and allowed to stand for 5 to 12 days.

(154) The method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus according to (153), wherein, after an extraction solvent is added to a crushed inflamed tissues and allowed to stand for 3 to 7 days, it is warmed at 35 to 40°C with stirring for 3 to 4 days more.

(155) The method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus according to any of (152) to (154), wherein the extraction solvent to be added to the crushed inflamed tissues is a phenol solution.

(156) The method for manufacturing an extract from inflamed skins of rabbits inoculated with vaccinia virus according to (155), wherein the amount of the phenol solution is one- to five-fold of the amount of the crushed inflamed tissues.

(157) An extract from inflamed skins of rabbits inoculated with vaccinia virus which is manufactured by the manufacturing method according to any of (152) to (156).

(158) The extract from inflamed skins of rabbits inoculated with vaccinia virus according to (157), wherein the amount of N-acetylneuraminic acid contained in the extract is 4000 ng or more per unit of the extract.

(159) The extract from inflamed skins of rabbits inoculated with vaccinia virus according to (157), wherein the amount of N-acetylneuraminic acid contained in the extract is 4000 to 22500 ng per unit of the extract.

(160) A preparation containing the extract from inflamed skins of rabbits inoculated with vaccinia virus manufactured by the manufacturing method according to any of (152) to (156).

(161) The preparation according to (160), wherein the preparation is an injectable preparation.

(162) The preparation according to (160), wherein the preparation is a 3 mL injectable preparation.

(163) The preparation according to (160), wherein the preparation is a tablet.

(164) The preparation according to (160), wherein the amount of N-acetylneuraminic acid contained in the preparation is 4000 ng or more per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus contained in the preparation.

(165) The preparation according to (160), wherein the amount of N-acetylneuraminic acid contained in the preparation is 4000 to 22500 ng per unit of the extract from inflamed skins of rabbits inoculated with vaccinia virus contained in the preparation.

(166) The preparation according to (161), wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is 4800 ng or more per mL of the injectable preparation.

(167) The preparation according to (161), wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is 4800 to 27000 ng per mL of the injectable preparation.

(168) The preparation according to (162), wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is 14400 ng or more per ampoule of the injectable preparation.

(169) The preparation according to (162), wherein the amount of N-acetylneuraminic acid contained in the injectable preparation is 14400 to 81000 ng per ampoule of the injectable preparation.

(170) The preparation according to (163), wherein the amount of N-acetylneuraminic acid contained in the tablet is 16000 ng or more per tablet.

(171) The preparation according to (163), wherein the amount of N-acetylneuraminic acid contained in the tablet is 16000 to 90000 ng per tablet.

[Industrial Applicability]

[0073]

As mentioned hereinabove, the present invention provides an extract from inflamed skins of rabbits inoculated with vaccinia virus or a preparation containing the extract which is deemed to be appropriately manufactured by such a means that the content of N-acetylneuraminic acid is measured so that the fact of containing a predetermined amount therein is confirmed. The present invention also provides an inspection method by which the extract and the preparation is deemed to be appropriately manufactured by means of confirming that each manufacturing lot of the extract contains a predetermined amount of N-acetylneuraminic acid. Since the extract and the preparation as such are manufactured using biological tissues, it is now possible to warrant the quality thereof for each lot in more strictly whereby that is very useful.

## CLAIMS

1. A process for reducing the variations in the effect of a pharmaceutical preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, comprising measuring the amount of N-acetylneuraminic acid contained in the pharmaceutical preparation of each manufacturing lot and confirming the content thereof to be
  - (i) if the pharmaceutical preparation is an injectable preparation, 4800 ng or more per ml of the liquid extract present in the pharmaceutical preparation, or
  - (ii) if the pharmaceutical preparation is a tablet, 16000 ng or more per tablet of the pharmaceutical preparation.
2. The process of claim 1, wherein the amount of N-acetylneuraminic acid in the injectable preparation is 4800-27000 ng per ml of the injectable preparation.
3. The process of claim 1, wherein the amount of N-acetylneuraminic acid in the tablet is 16000-90000 ng per tablet.
4. The process of any of claims 1-3, wherein the pharmaceutical preparation is an analgesic agent.
5. The process of claim 4, wherein the analgesic agent is a therapeutic agent for non-inflammatory pain disease.
6. Use of a pharmaceutical preparation containing an extract from inflamed skins of rabbits inoculated with vaccinia virus, wherein the amount of N-acetylneuraminic acid contained in the pharmaceutical preparation of

each manufacturing lot and confirming the content thereof is measured to be

(i) if the pharmaceutical preparation is an injectable preparation, 4800 ng or more per ml of the liquid extract present in the pharmaceutical preparation, or

(ii) if the pharmaceutical preparation is a tablet, 16000 ng or more per tablet of the pharmaceutical preparation;

in the manufacture of a medicament for reducing the variations in the effect of the pharmaceutical preparation in a subject.