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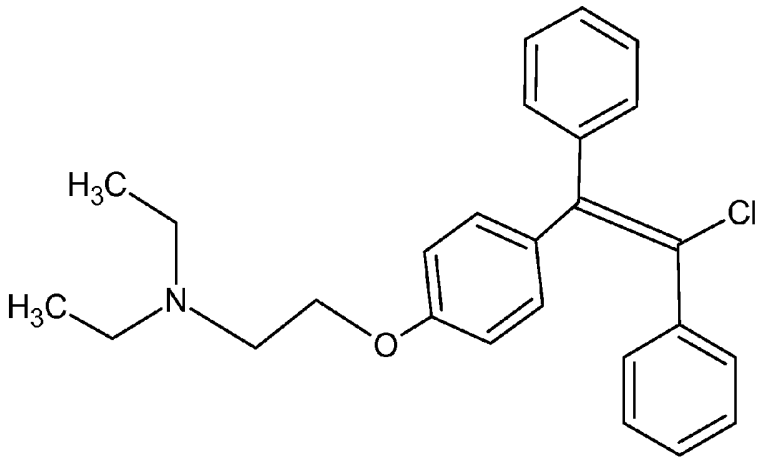
[Continued on next page]

(54) Title: TRANS-CLOMIPHENE FORMULATIONS AND USES THEREOF

Fig. 1



(57) Abstract: The present invention provides iraws-clomiphene and pharmaceutically acceptable salts and solvates thereof, characterized in that *trans* -clomiphene is in particulate form having a specific size range. The invention is also directed to pharmaceutical compositions comprising or formulated using trans -clomiphene or pharmaceutically acceptable satls and solvates having a specified size range and their use in treating disorders including secondary hypogonadism, type 2 diabetes, elevated cholesterol, elevated triglycerides, wasting, lipodystrophy, female and male infertility, benign prostate hypertrophy, prostate cancer, breast cancer, ovarian cancer and endometrial cancer.

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TRANS-CLOMIPHENE FORMULATIONS AND USES THEREOF

FIELD OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/691,722, filed August 21, 2012, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to trans-clomiphene, in particulate form, useful for treatment of various hormone-dependent disorders. More particularly, the trans-clomiphene is of a particle size range that provides an enhanced bioavailability.

BACKGROUND

[0003] Clomiphene is a selective estrogen receptor modulator related to tamoxifen. Clomiphene binds to estrogen receptors and blocks the normal estrogen feedback on the hypothalamus and subsequent negative feedback on the pituitary. This leads to increases in luteinizing hormone (LH) and follicle stimulating hormone (FSH). In men, these increased levels of gonadotropins stimulate the Leydig cells of the testes and result in the production of higher testosterone levels. For example, Tenover *et al.*, J. Clin. Endocrinol. Metab. *64*:1103, (1987) and Tenover *et al.*, J. Clin. Endocrinol. Metab. *64*:1118 (1987) found increases in FSH, LH in both young and old men after treatment with clomiphene. They also found increases in free and total testosterone in men with young men showing significant increases.

[0004] In females, clomiphene is currently approved as a mixture of both cis- and trans-isomers, the cis-isomer being present as about 30% to 50% (Merck Manual) for the induction of ovulation in anovulatory women. The increases in LH and FSH in anovulatory females following administration of clomiphene result in follicular growth and ultimately ovulation. The drug is recommended to be administered for 5 days at a dose of up to 100 mg daily

[0005] Ernst *et al.*, J. Pharmaceut. Sci. *65*:148 (1976), have shown that clomiphene is a mixture of two geometric isomers which they refer to as *cis*,-Z-, clomiphene (*cis*-clomiphene or zuclomiphene) and *trans*-,E-, clomiphene, (*trans*-clomiphene or enclomiphene). According to Ernst, *et al.* *trans*-clomiphene HCI has a melting point of 149°C-150.5°C, while

cis-clomiphene HCI has a melting point of 156.5°C-158°C. Ernst *et al.* have also noted that (the trans-isomer) is antiestrogenic (AE) while the *cis*-isomer is the more potent and more estrogenic form and has also been reported to have anti-estrogenic activity. The authors attribute the effect of the drug on ovulatory activity to both forms stating that the mixture is more effective than *trans*-clomiphene alone. The trans-isomer aids ovulation at the level of the hypothalamus. The estrogenic isomer *cis*-clomiphene contributes to enhanced ovulation elsewhere in the physiologic pathway leading to ovulation. The isomers are also reported to have different *in vivo* half-life. The cis isomer has been reported to leave residual blood levels for in excess of one month following a single dose.

[0006] Clomiphene has been associated with numerous side effects including: blurred vision, abdominal discomfort, gynecomastia, testicular tumors, vasomotor flushes, nausea, and headaches. Furthermore, other studies suggest that clomiphene possesses both genotoxic and tumor enhancement effects. The net outcome of these observations is that clomiphene in its current format, having between 30% and 50% of the cis isomer, would be unacceptable for chronic therapy in men for the treatment of testosterone deficiency.

[0007] Oral administration of trans-isomer of clomiphene (trans-clomiphene or enclomiphene) has been demonstrated to be effective in the treatment of a panoply of disorders ranging from secondary hypogonadism in males to induction of ovulation in anovulatory females. An improvement in the physical characteristics of trans-clomiphene would potentially offer a more beneficial therapy.

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SUMMARY

[0008] The present invention provides trans-clomiphene, characterized in that the trans-clomiphene is in particulate form, said particles having a mean particle size of less than about **30** microns, and preferably between about **5** and **20** microns.

[0009] Further, the present invention encompasses trans-clomiphene, wherein at least 90% of the particles have a particles size of less than about 50 microns.

[0010] Pharmaceutical compositions comprising or formulated using trans-clomiphene in particulate form are also provided. The compositions are used for treating a variety of disorders including, without limitation, secondary hypogonadism, type 2 diabetes, elevated cholesterol, elevated triglycerides, wasting, lipodystrophy, female and male infertility, benign prostate hypertrophy, prostate cancer, breast cancer, uterine cancer and ovarian cancer.

BRIEF DESCRIPTION OF THE DRAWING

[0011] FIG. 1 shows the chemical structure of *trans*-clomiphene.

DETAILED DESCRIPTION

[0012] While the present invention is capable of being embodied in various forms, the description below of several embodiments is made with the understanding that the present disclosure is to be considered as an exemplification of the invention, and is not intended to limit the invention to the specific embodiments illustrated. Headings are provided for convenience only and are not to be construed to limit the invention in any way. Embodiments illustrated under any heading may be combined with embodiments illustrated under any other heading.

[0013] It is to be understood that any ranges, ratios and ranges of ratios that can be formed by any of the numbers or data present herein represent further embodiments of the present invention. This includes ranges that can be formed that do or do not include a finite upper and/or lower boundary. Accordingly, the skilled person will appreciate that many such ratios, ranges and ranges of ratios can be unambiguously derived from the data and numbers presented herein and all represent embodiments of the invention.

[0014] Before the present compounds, compositions and methods are disclosed and described, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the present specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0015] The term "oral" administration means that the active agent is in a formulation designed to be ingested, i.e. designed to be delivered to the gastrointestinal system for absorption.

[0016] The term "effective dosage" means an amount of the composition's active component sufficient to treat a particular condition.

[0017] The term "treat" or "treatment" as used herein refers to any treatment of any progesterone-dependent disorder or disease, and includes, but is not limited to, inhibiting the disorder or disease arresting the development of the disorder or disease; relieving the disorder or disease, for example, causing regression of the disorder or disease; or relieving the condition caused by the disease or disorder, relieving the symptoms of the disease or disorder.

[0018] The term "prevent" or "prevention," in relation to a progesterone-dependent disorder or disease, means preventing the onset of disorder or disease development if none had occurred, or preventing further disorder or disease development if the disorder or disease was already present.

[0019] The term "pharmaceutically acceptable salt" refers to a salt prepared from a pharmaceutically acceptable non-toxic inorganic or organic acid. Inorganic acids include, but are not limited to, hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, and phosphoric. Organic acids include, but are not limited to, aliphatic, aromatic, carboxylic, and sulfonic organic acids including, but not limited to, formic, acetic, propionic, succinic, benzoic camphorsulfonic, citric, fumaric, gluconic, isethionic, lactic, malic, mucic, tartaric, para-toluenesulfonic, glycolic, glucuronic, maleic, furoic, glutamic, benzoic, anthranilic, salicylic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic, stearic, sulfanilic, alginic, and galacturonic acid. A preferred salt is the citrate salt.

[0020] The term "solvate" represents an aggregate that comprises one or more molecules of the solute, trans-clomiphene, with a molecule of solvent.

[0021] The term "mean particle size" is defined as equivalent spherical diameter as determined by laser light diffraction scattering.

[0022] In various embodiments, the present invention provides trans-clomiphene with a particle size within the specified narrow range. Control of the particle size within the specified narrow range provides a beneficial *in vivo* bioavailability.

[0023] The mean particle size of trans-clomiphene according to the present invention is less than about 30 microns, preferably between about 5 and about 20 microns. Further, the invention encompasses trans-clomiphene with at least 90% of the particles having a particle size of less than about 50 microns. More preferably, the mean particle size range is between about 5 and about 20 microns with at least 90% of the particles having a size of less than about 35 microns.

[0024] The invention also provides pharmaceutical compositions comprising or formulated using trans-clomiphene with a particle size within the specified narrow range and one or more pharmaceutically acceptable carriers.

[0025] Trans-clomiphene's chemical name is trans-2-(p-(2-chloro-1,2-diphenylvinyl)phenoxy)triethylamine (or 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine). The chemical structure is illustrated at Figure 1. "Trans-clomiphene" also encompasses the salts and solvates thereof, with the citrate salt being preferred. Trans-

clomiphene is a selective estrogen receptor modulator (SERM) which is believed to interfere at a hypothalamic level with steroid feedback inhibition of gonadotropin secretion thereby increasing the release of FSH and LH.

[0026] Trans-clomiphene can be made according to established procedures. U.S. Patent No. 2,914,563 describes the preparation of clomiphene and is incorporated herein by reference in its entirety. U.S. Patent Number 3,848,030 describes a method to separate the cis- and trans-isomers of clomiphene, and is incorporated herein by reference in its entirety.

[0027] Trans-clomiphene within a mean particle size of less than about 30 microns, preferably between about 5 and about 20 microns, can be administered by a variety of routes including but not limited to, intravenous, subcutaneous, buccal, transmucosal, intrathecal, intradermal, intracisternal, intramuscular, transdermal, intraperitoneal, epidural, vaginal, rectal, intranasal, sublingual, intra-articular, intra-cerebrospinal and intrasynovial, although, oral administration is the preferred route. Thus, another aspect of the present invention is a pharmaceutical composition comprising an effective amount of trans-clomiphene or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

[0028] Trans-clomiphene may be present in the pharmaceutical composition between 0.1% and 99.9% by weight of the formulation and may be the only active agent in the composition or may be combined with one or more additional active agents, depending on the intended use of the composition. The composition may comprise trans-clomiphene at a dosage between about one mg to about 200 mg (although the determination of optimal dosages is with the level of ordinary skill in the art). The composition may comprise trans-clomiphene at a dosage of about 1 mg, 2 mg, 3, mg, 4 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg or there between. Preferably, the composition comprises trans-clomiphene at a dosage of between 5 and 100 mg, e.g. at a dosage of 12.5 mg, 25 mg or 50 mg. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipient and salt must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0029] Pharmaceutical compositions of the present invention can be prepared by any procedure known in the art using readily available and well known ingredients. For example, trans-clomiphene can be formluated with common excipients, diluents or carriers and formed into tablets, capsules, suspensions, powders and the like. Examples of excipients, diluents

and carriers suitable for such formulations include, without limitation, filler and extenders such as strach, sugars, mannitol and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin and polyvinylpyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate, sodium bicarbonate and cross-linked povidone; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, polysorbate 80, glyercol monstearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate and solid polyethyl glycols.

[0030] The pharmaceutical compositions are useful to increase testosterone e.g. in secondary hypogonadal males to treat the secondary hypogonadism or to treat a disorder related thereto such as, without limitation, oligospermia, azoospermia, wasting and depression as described in US Patent No. 7,759,360, the entire content of which is hereby incorporated by reference. The pharmaceutical compositions are also useful to decrease cholesterol levels as described in US Patent No.7,368,480, the entire contents of which are hereby incorporated by reference. The pharmaceutical compositions can also be used to prevent or treat a condition selected from the group consisting of benign prostate hypertrophy, prostate cancer and elevated triglycerides as described in US Patent Application Publication No..2008/0242726, the entire contents of which are hereby incorporated by reference. The pharmaceutical compositions can also be used to prevent the transition from metabolic syndrome to type 2 diabetes or to treat type 2 diabetes or to reduce fasting glucose levels as described in US Patent Application Publication No. 2009/0099265, the entire contents of which are hereby incorporated by reference. The pharmaceutical compositions can also be used to treat female infertility in which case the composition is preferably administered to an anovulatory female as a daily dose in the early follicular phase of the menstrual cycle for five consecutive days. The pharmaceutical compositions are also useful for treating and/or preventing breast cancer and/or as an adjuvant therapy following initial treatment with surgery in order to minimize the possibility of relapse. The pharmaceutical compositions are also useful for treating endometrial (or uterine) cancer and ovarian cancer.

[0031] All of the references discussed herein are incorporated by reference in their entirety.

[0032] The following Examples are meant to be illustrative of the invention and are not intended to limit the scope of the invention as set out is the appended claims.

EXAMPLE 1

Preparation of Trans-Clomiphene Citrate

[0033] .Clomiphene citrate was prepared as follows:

[0034] A mixture of 20 g of 1-[p-(β-diethylaminoethoxy)phenyl]-1,2-diphenylethanol in 200 cc of ethanol containing an excess of hydrogen chloride was refluxed 3 hours. The solvent and excess hydrogen chloride were removed under vacuum, and the residue was dissolved in a mixture of ethyl acetate and methylene chloride. 1-[p-(β-diethylaminoethoxy)phenyl]-1,2-diphenylethylene hydrochloride was obtained, melting at 148 to 157 C. This hydrochloride salt was treated with N-chlorosuccinimide in dry chloroform under reflux. The product then obtained was converted to the free base and treated with citric acid. The di-hydrogen citrate salt of 1-[p-(β-diethylaminoethoxy)phenyl]-1,2-diphenylchloroethylene was obtained, melting at 116.5 to 118 C. Clomiphene citrate obtained by this process comprises between 30 and 50% cis-isomer and between 70 and 50% trans-isomer.

[0035] Trans-clomiphene was then separated from the racemic mixture of clomiphene isomers using the process described in Examples 31 and 32 of U.S. Patent Number 3,848,030.

EXAMPLE 2

Particle Size Analysis

[0036] Trans-clomiphene is characterized for size using an instrument adapted to measure equivalent spherical volume diameter, such as a Malvern Mastersizer 2000 laser diffraction particle size analyzer or equivalent instrument. After being characterized for size, the trans-clomiphene is then milled, if necessary, preferably using a pin mill under suitable conditions of mill rotation rate and feed rate, to bring the particle size value within the above mentioned limits according to the invention. The efficiency of milling is checked by sampling using a Malvern Mastersizer 2000 laser diffraction particle size analyzer and the final particle size is checked in a similar manner.

[0037] Trans-clomiphene in its particulate form within the above mentioned limits according to the invention may then be mixed with an excipient or carrier as necessary and for example used to fill capsules. Because the particles before or after milling are irregular in shape, it is necessary to characterize them by measurement of a property of the particles related to the sample property possessed by a theoretical spherical particle. The particles are thus allocated an "equivalent spherical diameter."

[0038] The values found from characterizing a large number of "unknown" particles can be plotted frequency vs. diameter. This gives a characteristic curve representing size distribution of the sample, i.e., cumulative percentage under size distribution curve. Values from this can be read off directly or plotted on log-probability paper to give an appropriate straight line. The mean equivalent spherical volume diameter is the 50% undersize value. The mean equivalent spherical volume diameter found is thus a statistical representation of a theoretical particle having the same property as the "unknown" particle. The following is a description by way of example.

[0039] The particle size of trans-clomiphene citrate from Example 1 was analyzed. The refractive index of trans-clomiphene citrate was measured microscopically suing the Becke line method as described in McCrone W.C. et al., Polarized Light Microscopy, McCrone Research Institute, Chicago, 1984, pages 126-127. The sample refractive index was estimated to be 1.62 using this method.

[0040] 0.1% (w/v) Lecithin in Isopar G™, 0.1% (w/v) Span™ 85 (sorbitane trioleate) in hexane, and 0.01% (w/v) Tween® 20 (polysorbate 20) in water were evaluated as dispersants for trans-clomiphene citrate. Samples suspended easily in each of the two organic dispersants and settled out of suspension slowly. Partial dissolution was observed in the sample suspended in aqueous dispersant, so 0.01% (w/v) Tween® 20 in water was unsuitable. Samples dispersed in the Isopar G™-based and hexane-based dispersants were examined microscopically. Sample composition and morphology were similar, revealing crystalline blades and needles between 10 and 150 μm in length, and differences in agglomeration were minor, with some soft agglomerates greater than 400 μm observed. 0.1% (w/v) Span™ 85 in hexane was selected for further analysis.

[0041] The sample absorption, or imaginary component of the refractive index, is a measure of the amount of light absorbed by the sample and is an important parameter in calculating a particle size distribution from a measured scattering pattern. The sample absorption cannot be measured experimentally so it must be estimated using "trial and error" using a scattering pattern for a particular compound. An initial particle size measurement was collected using the following parameters and particle size distributions were calculated from the scattering data using various sample absorption values:

Refractive Index	1.62
Default Particle Absorption	0.01
Sample Measurement Time	10 seconds

Background Measurement Time	20 seconds
Default Pump Speed	1000 rpm
Recirculation Time	60 seconds
Model	General Purpose
Sensitivity	Normal
Particle Shape	Irregular

[0042] The weighted residual is a measure of the goodness-of-fit between the measured data and a mathematical model that allows conversion of that data into a particle size distribution. A sample absorption index of 0.01 produced the best fit and was chosen for all subsequent particle size analyses.

[0043] Repeated particle size measurements of the sample dispersed in 0.1% (w/v) Span™ 85 in hexane were collected while recirculating over the course of approximately five minutes with a pump speed of 1000 rpm. Particle size (d10, d50, d90) was plotted versus the recirculation time. Values for both the d10 and d50 fell within a narrow range for the duration. The decrease in the d90 primarily occurred over the first 90 seconds with a gradual decrease thereafter. This decrease in the d90 suggested that agglomerates were dispersed within 90 seconds and some attrition may have occurred with longer recirculation so a recirculation time of 90 seconds was selected for further analyses.

[0044] Repeated particle size measurements of the sample were collected while recirculating for 90 seconds with increasing pump speeds to examine the effect of pump speed on particle size. Differences in the d10 and d50 were small, but the d90 showed an increase in particle size with a pump speed of 1500 rpm but no further increase at 2000 rpm. A pump speed of 1500 rpm was selected to optimize both dispersion of agglomerates and suspension of the larger particles and to minimize attrition of the blades and needles.

[0045] The suspended sample was recovered from the dispersion cell following particle size measurement with a pump speed of 1500 rpm and was microscopically examined. Primary particles were reasonably well dispersed and photomicrographs were consistent with those collected prior to recirculation with similar numbers and sizes of blades and needles suggesting that attrition had been minimized.

[0046] The repeatability of the method was evaluated by making five replicate measurements using the final method conditions. The relative standard deviations for the d10, d50 and d90

were 2.60%, 3.42% and 10.70% respectively. All fell within the USP recommendation of ≤ 30%, ≤ 10% and ≤ 15% for the d10, d50 and d90 respectively.

[0047] One particle size measurement of each sample of trans-clomiphene citrate was collected using the final method conditions. Three of the lots shared a similar bimodal particle size distribution whereas one lot contained a third mode consisting of much larger particles. The remaining lot of was bimodal but reflected much larger particle sizes than the distributions of the earlier three lots with bimodal distributions.

[0048] Photomicrographs of each lot of trans-clomiphene citrate dispersed in 0.1% (w/v) Span™ 85 in hexane were collected following particle size measurement using final method conditions. The results were consistent with the particle size results.

[0049] The three lots sharing a similar particle size distribution also shared similar morphology primarily consisting of blades and needles 10-150 μm in length and a few equant particles ≤10 μm. Final conditions for determining particle size were:

Sample Refractive Index	1.62
Sample Absorption	0.01
Dispersant	0.1% (w/v) Span 85 in hexane
Dispersant Refractive Index	1.39
Sample Measurement Time	10 seconds
Background Measurement Time	20 seconds
Pump Speed	1500 rpm
Recirculation Time	90 seconds
Model	General Purpose
Sensitivity	Normal
Particle Shape	Irregular

[0050] Refractive index determination was performed using a Leica DM LP microscope. A single, substage polarizer was used to view samples. Samples were placed on a glass slide, a coverslip was placed over the sample, and a drop of certified Cargill refractive index oil was added. The movement of the Becke line was observed while defocusing the sample.

[0051] Polarized light microscopy was performed using a Leica DM LP microscope equipped with a Spot Insight color camera. Crossed-polarized light was used with a first order red compensator. A 10x, 20x or 40x objective was used to view the sample. Images were acquired at ambient temperature using Spot Advanced software (v.4.5.9).

[0052] Particle size data was acquired using a Malvern Instruments MS2000 equipped with a Hydro 2000μP dispersion unit. Data was collected and analyzed using Mastersizer 2000 v. 5.60 software using volume based measurements. NIST-traceable glass beads were used as the reference standard.

[0053] Particle size of trans-clomiphene citrate using the final method conditions is reproduced below:

Lot No.	d10 (μm)[a]	d50 (μm)[b]	d90 (μm)[c]
31249	4.850	13.455	76.891
16204	8.058	106.743	318.464
24712	4.038	13.902	218.573
32305	3.373	9.664	44.995
24867	4.794	13.418	70.289

a. 10% of the total volume of particles is less than the indicated particle size
b. 50% of the total volume of particles is less than the indicated particle size
c. 90% of the total volume of particles is less than the indicated particle size

[0054] The particle size distribution of trans-clomiphene from Lot No. 32305 is reproduced below:

Size (μm)	Vol under %	Size (μm)	Vol under %	Size (μm)	Vol under %	Size (μm)	Vol. under %	Size (μm)	Vol. under %
0.010	0.00	0.105	0.00	1.096	0.10	11.482	57.87	120.226	99.04
0.011	0.00	0.120	0.00	1.259	0.28	13.183	63.80	138.038	99.72
0.013	0.00	0.138	0.00	1.445	0.60	15.136	69.20	158.489	99.97
0.015	0.00	0.158	0.00	1.660	1.12	17.378	73.95	181.970	100.00
0.017	0.00	0.182	0.00	1.905	1.93	19.953	77.98	208.930	100.00
0.020	0.00	0.209	0.00	2.188	3.10	22.909	81.29	239.883	100.00
0.023	0.00	0.240	0.00	2.512	4.72	26.303	83.92	275.423	100.00
0.026	0.00	0.275	0.00	2.884	6.86	30.200	85.99	316.228	100.00
0.030	0.00	0.316	0.00	3.311	9.59	34.674	87.62	363.078	100.00
0.035	0.00	0.363	0.00	3.802	12.94	39.811	88.95	416.869	100.00
0.040	0.00	0.417	0.00	4.385	16.94	45.709	90.13	478.630	100.00
0.046	0.00	0.479	0.00	5.012	21.58	52.481	91.28	549.541	100.00
0.052	0.00	0.550	0.00	5.754	26.82	60.256	92.50	630.957	100.00
0.060	0.00	0.631	0.00	6.607	32.58	69.183	93.84	724.438	100.00

Size (μm)	Vol under %	Size (μm)	Vol under %	Size (μm)	Vol under %	Size (μm)	Vol. under %	Size (μm)	Vol. under %
0.069	0.00	0.724	0.00	7.586	38.74	79.433	95.28	831.764	100.00
0.079	0.00	0.832	0.00	8.710	45.14	91.201	96.72	954.993	100.00
0.091	0.00	0.965	0.02	10.00	51.59	104.713	98.02	1096.478	100.00

[0055] Trans-clomiphene with the particle size distribution of the invention is expected to provide a consistent and improved *in vivo* absorption/bioavailability profile compared with trans-clomiphene having a particle size distribution outside the specified range. In addition to ensuring consistent delivery of trans-clomiphene to, and absorption from, the gastrointestinal tract, the specified particle size distribution provides better control during the manufacturing process. Controlling the particle size also minimizes variations in the quantity of water required to bring about the desired granulation.

[0056] Trans-clomiphene with the particle size distribution of the invention, alone or in combination with another active agent, generally will be administered in a convenient formulation. The following formulations are only illustrative and not intended to limit the scope of the invention.

EXAMPLE 3

Formulations

[0057] .Gelatin capsules comprising trans-clomiphene are prepared using the following:

Component	Quantity (mg/capsule)
Trans-clomiphene citrate	5.0-100
Microcrystalline cellulose	0-343.2
Magnesium Stearate	0-8-

[0058] Trans-clomiphene, in crystal form, is blended with 1/3 of the total microcrystalline cellulose and passed through a mesh screen to ensure good distribution of the materials. The remaining 2/3 of the microcrystalline cellulose is then passed through a mesh screen and blended with the powder mixture. The resulting mixture is then milled through a suitable milling machine (e.g. a Comil® mill). Magnesium stearate, previously passed through a mesh screen, is added and mixed with the resulting granules. Following a uniformity

analysis, the resulting mixture is encapsulated into gelatin capsules. A preferred gelatin capsule (size 3) formulation follows:

Component	**Quantity (mg/capsule)**
Trans-clomiphene citrate	12.5
Microcrystalline cellulose	85.5
Magnesium Stearate	2.0
TOTAL	100.0

[0059] Combination capsules comprising trans-clomiphene and an additional active agent (e.g. an aromatase inhibitor or oral testosterone) may be prepared according to the methods above:

Component	**Quantity (mg/capsule)**
Trans-clomiphene citrate	12.5
Anastrazole	1.0-50.0
Microcrystalline cellulose	85.5
Magnesium Stearate	2.0
TOTAL	100.0

Component	**Quantity (mg/capsule)**
Trans-clomiphene citrate	12.5
Testosterone Undecanoate	80-200mg
Microcrystalline cellulose	85.5
Magnesium Stearate	2.0

[0060] Alternatively, tablets each containing 5.0 to 100 mg trans-clomiphene can be made as follows:

Component	**Quantity (mg/tablet)**
Trans-clomiphene citrate	5.0-100
Starch	30-60
Polyvinylpyyrolidone	0-8
Microcrystalline cellulose	25-45
Magnesium Stearate	0.1-2.0
Talc	0-3

[0061] For tablet formulations, trans-clomiphene, starch and cellulose are passed through a mesh sieve (e.g. No. 45) and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powder which is then passed through a mesh sieve (e.g. No. 14). The granules produced are dried at 50-60 C and passed through a mesh sieve (e.g. No. 18). The magnesium stearate and talc, previously passed through a sieve (e.g. NO. 6) are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

[0062] Alternatively, suspensions each containing between 1.0 and 100 mg of trans-clomiphene per 5 ml dose are made as follows:

Component	**Quantity (mg/5 ml)**
Trans-clomiphene citrate	1.0-100 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 ml
Flavor	q.v.
Color	q.v.
Purified water to	5 ml

[0063] For suspensions, trans-clomiphene is passed through a mesh sieve (e.g. No. 45) and mixed with the sodium carboxymethyl cellulose and syrup to form smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

[0064] Alternatively, suppositories may be prepared as follows:

Component	**Quantity (mg/suppository)**
Trans-clomiphene citrate	100-500
Saturated fatty acid glycerides	1000-3000

[0065] For suppositories, trans-clomiphene is passed through a mesh sieve (e.g. No. 60) and suspended in the saturated fatty acid glycerides previously melted using minimal heat necessary. The mixture is then poured into a suppository mold and allowed to cool.

CLAIMS

1. The compound trans-clomiphene and pharmaceutically acceptable salts and solvates thereof, characterized in that the compound is in particulate form, said particles having a mean particle size of less than about 30 microns, at least about 90% of said particles have a size of less than about 50 microns.

2. The compound of claim 1 wherein said particles have a mean particle size of between about 5 and about 20 microns.

3. A compound of claim 1 which is a non-solvated crystalline form.

4. The compound of any of claims 1-3 wherein the compound is trans-clomiphene citrate.

5. A pharmaceutical formulation comprising or formulated using the compound of claim 1 and one or more pharmaceutically acceptable carriers, diluents or excipients.

6. The pharmaceutical formulation according to claim 5 wherein the compound is trans-clomiphene citrate.

7. The pharmaceutical formulation according to claim 5 or 6 wherein the formulation is a capsule.

8. The pharmaceutical formulation according to claim 7 comprising about 5 to about 100 mg trans-clomiphene citrate.

9. The pharamceutical formulation according to claim 8 comprising 12.5 mg, 25 mg or 50 mg trans-clomiphene citrate.

10. The pharmaceutical formulation according to any one of claims 5-9, further comprising microcrystalline cellulose and/or magnesium stearate.

11. The pharmaceutical formulation according to claim 10, each capsule comprising about 12.5 mg trans-clomiphene citrate, about 85.5 mg microcrystalline cellulose and about 2.0 mg magnesium stearate.

12. The pharmaceutical formulation according to any of claims 5-11, for use in treating secondary hypogonadism in a human male or for treating a disorder associated therewith.

13. The pharmaceutical formulation for use according to claim 12, wherein the disorder associated with secondary hypogonadism is selected from reduction of muscle mass, reduction of bone density, reduction of libido, oligospermia, and azoospermia.

14. The pharmaceutical formulation according to any of claims 5-11 for use in treating infertility in a human female.

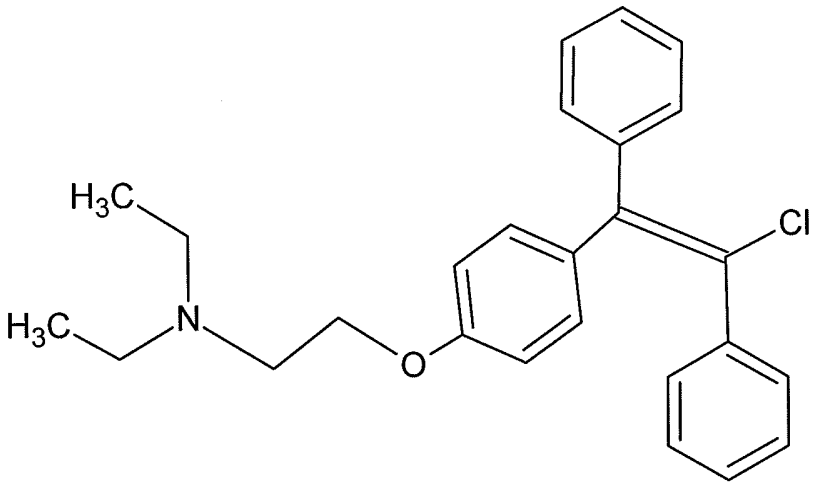
15. The pharmaceutical formulation for use according to claim 14, wherein the formulation is administered to an anovulatory female as a daily dose for a period of five consecutive days.

16. The pharmaceutical formulation according to any of claims 5-11 for use in a method for treating and/or preventing type 2 diabetes in a human male.

17. The pharmaceutical formulation according to any of claims 5-11 for treating or preventing breast cancer in a human female

18. The pharmaceutical formulation according to any of claims 5-11 for treating endometrial, uterine or ovarian cancer in a human female.

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/032659

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/054248 A1 (REPROS THERAPEUTICS INC [US]; VAN AS ANDRE [US]) 14 May 2010 (2010-05-14) paragraphs [0015] - [0017], [0021] paragraphs [0041], [0045], [0061], [0073] - [0083] paragraphs [0088], [0104], [0106] claims 1-4 figures 2, 4	1-18
A	US 2 914 563 A (ALLEN ROBERT E ET AL) 24 November 1959 (1959-11-24) cited in the application the whole document	1-18

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2013	19/07/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weiss, Marie-France

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/032659

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010054248 A1	14-05-2010	AR 074302 A1	05-01-2011
		TW 201031400 A	01-09-2010
		WO 2010054248 A1	14-05-2010
US 2914563 A	24-11-1959	NONE	

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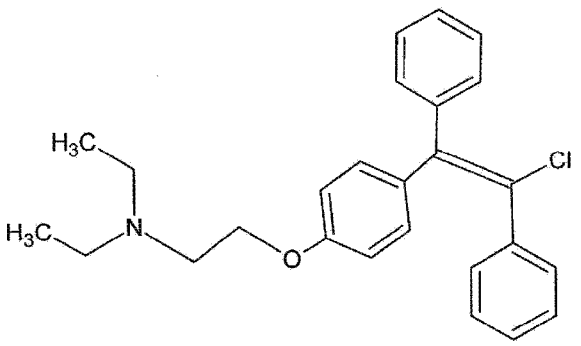
权利要求书1页 说明书9页 附图1页

(54)发明名称

反式克罗米酚(trans-clomiphene)调配物和其用途

(57)摘要

本发明提供反式克罗米酚和其药学上可接受的盐和溶剂合物,其特征在于反式克罗米酚呈具有特定粒度范围的微粒形式。本发明也涉及包含具有指定粒度范围的反式克罗米酚或药学上可接受的盐和溶剂合物或使用其调配的医药组合物和所述医药组合物用于治疗包括以下的病症的用途:继发性性腺功能低下症、2型糖尿病、高胆固醇、高三酸甘油酯、消瘦、脂质营养不良、女性和男性不孕症、良性前列腺肥大、前列腺癌、乳癌、卵巢癌和子宫内膜癌。



CN 104582686 A

1. 一种化合物反式克罗米酚和其药学上可接受的盐和溶剂合物，其特征在于所述化合物呈散粒形式，所述粒子具有小于约 30 微米的平均粒度，至少约 90％的所述粒子具有小于约 50 微米的粒度。

2. 根据权利要求 1 所述的化合物，其中所述粒子具有介于约 5 与约 20 微米之间的平均粒度。

3. 根据权利要求 1 所述的化合物，其是非溶剂化结晶形式。

4. 根据权利要求 1 到 3 中任一权利要求所述的化合物，其中所述化合物是反式克罗米酚柠檬酸盐。

5. 一种医药调配物，其包含根据权利要求 1 所述的化合物和一或多种药学上可接受的载剂、稀释剂或赋形剂或使用其调配。

6. 根据权利要求 5 所述的医药调配物，其中所述化合物是反式克罗米酚柠檬酸盐。

7. 根据权利要求 5 或 6 所述的医药调配物，其中所述调配物是胶囊。

8. 根据权利要求 7 所述的医药调配物，其包含约 5 到约 100mg 反式克罗米酚柠檬酸盐。

9. 根据权利要求 8 所述的医药调配物，其包含 12.5mg、25mg 或 50mg 反式克罗米酚柠檬酸盐。

10. 根据权利要求 5 到 9 中任一权利要求所述的医药调配物，其进一步包含微晶纤维素和 / 或硬脂酸镁。

11. 根据权利要求 10 所述的医药调配物，每一个胶囊包含约 12.5mg 反式克罗米酚柠檬酸盐、约 85.5mg 微晶纤维素和约 2.0mg 硬脂酸镁。

12. 根据权利要求 5 到 11 中任一权利要求所述的医药调配物，其用于治疗人类男性中的继发性性腺功能低下症或用于治疗与此相关的病症。

13. 根据权利要求 12 所述使用的医药调配物，其中与继发性性腺功能低下症相关的所述病症选自肌肉质量减小、骨密度减小、性欲降低、精子稀少症和无精子症。

14. 根据权利要求 5 到 11 中任一权利要求所述的医药调配物，其用于治疗人类女性中的不孕症。

15. 根据权利要求 14 所述使用的医药调配物，其中所述调配物持续连续 5 天的时段以日剂量形式投与向无排卵女性投与。

16. 根据权利要求 5 到 11 中任一权利要求所述的医药调配物，其用于治疗和 / 或预防人类男性中的 2 型糖尿病的方法中。

17. 根据权利要求 5 到 11 中任一权利要求所述的医药调配物，其用于治疗或预防人类女性中的乳癌。

18. 根据权利要求 5 到 11 中任一权利要求所述的医药调配物，其用于治疗人类女性中的子宫内膜癌、子宫癌或卵巢癌。

反式克罗米酚(trans-clomiphene)调配物和其用途

[0001] 相关申请的交叉引用

[0002] 本申请主张2012年8月21日提交的美国临时申请第61/691,722号的权益,其内容以引用的方式并入本文中。

技术领域

[0003] 本发明涉及适用于治疗各种激素依赖性病症的微粒形式的反式克罗米酚(trans-clomiphene)。更确切地说,反式克罗米酚具有提供增强的生物可用性的粒度范围。

背景技术

[0004] 克罗米酚是与他莫昔芬(tamoxifen)相关的选择性雌激素受体调节剂。克罗米酚结合到雌激素受体并且阻断下丘脑上的正常雌激素反馈和垂体上的后续负反馈。这导致促黄体激素(LH)和促卵泡激素(FSH)的增加。在男性中,这些增加的促性腺激素水平刺激睾丸的雷迪格细胞(Leydig cell)并且导致产生较高睾酮水平。举例来说,特诺弗(Tenover)等人,临床内分泌与代谢杂志(J.Clin.Endocrinol.Metab.)64:1103,(1987)和特诺弗等人,临床内分泌与代谢杂志64:1118(1987)发现在用克罗米酚治疗之后,年轻男性和老年男性的FSH、LH的增加。他们还发现男性自由和总睾酮的增加,其中年轻男性显示显著增加。

[0005] 在女性中,当前将克罗米酚批准为顺式和反式异构体的混合物,其中顺式异构体以约30%到50%存在(默克手册(Merck Manual)),用于诱导无排卵女性的排卵。无排卵女性在投与克罗米酚后的LH和FSH的增加导致卵泡生长和最终排卵。推荐持续5天以每天至多100mg的剂量投与药物。

[0006] 恩斯特(Ernst)等人,药学科学杂志(J.Pharmaceut.Sci.)65:148(1976)已显示克罗米酚为两种几何异构体的混合物,其将所述几何异构体称作顺式-Z-克罗米酚(顺式克罗米酚或珠氯米芬(zuclomiphene))和反式-E-克罗米酚(反式克罗米酚或恩氯米芬(enclomiphene))。根据恩斯特等人,反式克罗米酚HCI具有149℃-150.5℃的熔点,而顺式克罗米酚HCI具有156.5℃-158℃的熔点。恩斯特等人也已指出(反式异构体)是抗雌激素(AE)的,而顺式异构体是更强力的并更具雌激素形式并且也已报导具有抗雌激素活性。作者将药物对排卵活性的作用归因于两种形式,陈述混合物比单独的反式克罗米酚更有效。反式异构体在下丘脑水平帮助排卵。雌激素异构体顺式克 罗米酚在导致排卵的生理途径中的其它地方促进排卵增强。据报导异构体也具有不同体内半衰期。已报导顺式异构体在单剂量之后超过一个月保留残留的血液水平。

[0007] 克罗米酚已与许多副作用相关,所述副作用包括:视力模糊、腹部不适、男性乳房发育症、睾丸肿瘤、血管舒张潮红、恶心和头痛。此外,其它研究表明克罗米酚具有遗传毒性和肿瘤增强效应。这些观测的净结果是,具有介于30%与50%之间的顺式异构体的当前形式的克罗米酚对于慢性治疗男性以治疗睾酮缺乏将是不可接受的。

[0008] 已展示经口投与克罗米酚的反式异构体(反式克罗米酚或恩氯米芬)在治疗介于

男性的继发性性腺功能低下症到诱导无排卵女性的排卵范围内的形形色色的病症中有效。反式克罗米酚的物理特性的改进将潜在地提供更有益的治疗。

发明内容

[0009] 本发明提供反式克罗米酚，其特征在于所述反式克罗米酚呈微粒形式，所述粒子具有小于约 30 微米，并且优选地介于约 5 与 20 微米之间的平均粒度。

[0010] 另外，本发明涵盖反式克罗米酚，其中至少 90％的粒子具有小于约 50 微米的粒度。

[0011] 也提供包含微粒形式的反式克罗米酚或使用其调配的医药组合物。组合物用于治疗多种病症，包括（但不限于）继发性性腺功能低下症、2 型糖尿病、高胆固醇、高三酸甘油酯、消瘦、脂质营养不良、女性和男性不孕症、良性前列腺肥大、前列腺癌、乳癌、子宫癌和卵巢癌。

附图说明

[0012] 图 1 显示反式克罗米酚的化学结构。

具体实施方式

[0013] 尽管本发明能够以各种形式实施，但在理解应将本发明视为本发明的范例的情况下在下文中对若干实施例进行描述，并且并不打算将本发明限制为所说明的特定实施例。仅仅为方便起见而提供标题，并且不应理解为以任何方式限制本发明。在任何标题下说明的实施例可以与在任何其它标题下说明的实施例组合。

[0014] 应理解，可以由本文中呈现的数字或数据中的任一个形成的任何范围、比率和比率范围代表本发明的其它实施例。这包括可以形成的包括或不包括有限上边界和 / 或下边界的范围。因此，技术人员将理解，许多此类比率、范围和比率范围可以明确地衍生自本文中呈现的数据和数字并且全部代表本发明的实施例。

[0015] 在揭示和描述本发明化合物、组合物和方法之前，应理解本文中所用的术语仅仅出于描述具体实施例的目的并且不打算为限制性的。必须指出，除非上下文另外明确指示，否则如本说明书和所附权利要求书中所用，单数形式“一 (a/an)”和“所述 (the)”包括多个指示物。

[0016] 术语“经口”投药意味着活性剂呈被设计成用于摄取，即被设计成用于传递到胃肠系统中以供吸收的调配物形式。

[0017] 术语“有效剂量”意味着足以治疗特定病状的组合物的活性组分的量。

[0018] 如本文所用的术语“治疗 (treat/treatment)”是指任何孕酮依赖性病症或疾病的任何治疗，并且包括（但不限于）抑制病症或疾病，遏制病症或疾病的发展；缓解病症或疾病，例如引起病症或疾病的消退；或缓解由疾病或病症造成的病状，缓解疾病或病症的症状。

[0019] 关于孕酮依赖性病症或疾病的术语“预防 (prevent/prevention)”意味着在未出现病症或疾病发展时预防病症或疾病发展的起始，或在已存在病症或疾病时预防进一步的病症或疾病发展。

[0020] 术语“药学上可接受的盐”是指从药学上可接受的无毒无机酸或有机酸制备的盐。无机酸包括（但不限于）盐酸、氢溴酸、氢碘酸、硝酸、硫酸和磷酸。有机酸包括（但不限于）脂肪酸、芳香酸、羧酸和磺酸有机酸，包括（但不限于）甲酸、乙酸、丙酸、丁二酸、苯甲酸、樟脑磺酸、柠檬酸、反丁烯二酸、葡萄糖酸、羟乙基磺酸、乳酸、苹果酸、粘液酸、酒石酸、对甲苯磺酸、乙醇酸、葡糖醛酸、顺丁烯二酸、糠酸、谷氨酸、苯甲酸、邻氨基苯甲酸、水杨酸、苯乙酸、扁桃酸、亚甲基双羟萘酸 (embonic acid/pamoic acid)、甲磺酸、乙磺酸、泛酸、苯磺酸、硬脂酸、对氨基苯磺酸、褐藻酸和半乳糖醛酸。优选的盐是柠檬酸盐。

[0021] 术语“溶剂合物”表示包含一或多个溶质反式克罗米酚分子与溶剂分子的聚集体。

[0022] 术语“平均粒度”定义为如通过激光衍射散射测定的当量球径。

[0023] 在不同实施例中，本发明提供具有处于指定狭窄范围内的粒度的反式克罗米酚。将粒度控制在指定狭窄范围内提供有益的体内生物可用性。

[0024] 根据本发明的反式克罗米酚的平均粒度小于约 30 微米，优选地介于约 5 与约 20 微米之间。另外，本发明涵盖至少 90％的粒子具有小于约 50 微米的粒度的反式克罗米酚。更优选地，平均粒度范围介于约 5 与约 20 微米之间，并且至少 90％的粒子具有小于约 35 微米的粒度。

[0025] 本发明还提供包含具有处于指定狭窄范围内的粒度的反式克罗米酚和一或多种药学上可接受的载剂或使用其调配的医药组合物。

[0026] 反式克罗米酚的化学名称为反 -2-（对 -(2- 氯 -1,2- 二苯基乙烯基）苯氧基）三乙胺（或 2-[4-(2- 氯 -1,2- 二苯基乙烯基）苯氧基]-N,N- 二乙基乙胺）。化学结构说明于图 1 中。“反式克罗米酚”也涵盖其盐和溶剂合物，其中柠檬酸盐是优选的。反式克罗米酚是一种选择性雌激素受体调节剂 (SERM)，其被认为在下丘脑水平干扰促性腺激素分泌的类固醇反馈抑制，进而增加 FSH 和 LH 的释放。

[0027] 可以根据已确立的程序制得反式克罗米酚。美国专利第 2,914,563 号描述克罗米酚的制备并且以全文引用的方式并入本文中。美国专利第 3,848,030 号描述一种分离克罗米酚的顺式和反式异构体的方法，并且以全文引用的方式并入本文中。

[0028] 可以通过多种途径投与平均粒度小于约 30 微米，优选地介于约 5 与约 20 微米之间的反式克罗米酚，所述途径包括（但不限于）静脉内、皮下、颊内、经粘膜、鞘内、皮内、脑池内、肌内、经皮、腹膜内、硬膜外、经阴道、经直肠、鼻内、舌下、关节内、脑脊髓内和滑膜内，尽管经口投与是优选的途径。因此，本发明的另一方面是一种医药组合物，其包含有效量的反式克罗米酚或其药学上可接受的盐和药学上可接受的载剂、稀释剂或赋形剂。

[0029] 反式克罗米酚可以按调配物的重量计介于 0.1％与 99.9％之间存在于医药组合物中，并且取决于组合物的预定用途，其可以是组合物中的唯一活性剂或可以与一或多种其它活性剂组合。组合物可以包含剂量介于约 1mg 到约 200mg 之间的反式克罗米酚（尽管最佳剂量的测定在所属领域的一般技术水平内）。组合物可以包含剂量为约 1mg、2mg、3mg、4mg、5mg、10mg、15mg、20mg、25mg、30mg、35mg、40mg、45mg、50mg、55mg、60mg、65mg、70mg、75mg、80mg、85mg、90mg、95mg、100mg、110mg、120mg、130mg、140mg、150mg、160mg、170mg、180mg、190mg、200mg 或在其之间的反式克罗米酚。优选地，组合物包含剂量介于 5 与 100mg 之间，例如剂量为 12.5mg、25mg 或 50mg 的反式克罗米酚。「药学上可接受」意味着载剂、稀释剂、赋形剂和盐必须与调配物的其它成分相容并且对其接受者无害。

[0030] 可以使用可易于获得并且众所周知的成分通过所属领域中已知的任何程序制备本发明的医药组合物。举例来说，反式克罗米酚可以与常用赋形剂、稀释剂或载剂一起调配并且形成为片剂、胶囊、悬浮液、散剂等。适用于所述调配物的赋形剂、稀释剂和载剂的实例包括（但不限于）填充剂和增量剂，如淀粉、糖、甘露醇和硅衍生物；结合剂，如羧甲基纤维素和其它纤维素衍生物、藻酸盐、明胶和聚乙烯吡咯烷酮；保湿剂，如甘油；崩解剂，如碳酸钙、碳酸氢钠和交联聚维酮；用于延迟溶解的试剂， 如石蜡；吸收促进剂，如四级铵化合物；表面活性剂，如十六醇、聚山梨醇酯 80、单硬脂酸甘油酯；吸附载剂，如高岭土和膨润土；和润滑剂，如滑石、硬脂酸钙和硬脂酸镁和固体聚乙二醇。

[0031] 医药组合物适用于在例如继发性性腺功能低下男性中增加睾酮以治疗继发性性腺功能低下症或治疗与其相关的病症，如（但不限于）精子稀少症、无精子症、消瘦和抑郁症，其如美国专利第 7,759,360 号中所述，所述专利的全部内容以引用的方式并入本文中。医药组合物也适用于降低胆固醇水平，其如美国专利第 7,368,480 号中所述，所述专利的全部内容以引用的方式并入本文中。医药组合物也可以用于预防或治疗选自由以下组成的群组的病状：良性前列腺肥大、前列腺癌和高三酸甘油酯，其如美国专利申请公开第 2008/0242726 号中所述，所述专利的全部内容以引用的方式并入本文中。医药组合物也可以用于预防从代谢综合征到 2 型糖尿病的过渡或治疗 2 型糖尿病或降低空腹血糖水平，其如美国专利申请公开第 2009/0099265 号中所述，所述专利的全部内容以引用的方式并入本文中。医药组合物也可以用于治疗女性不孕症，在此情况下，优选地在月经周期的早卵泡期中连续 5 天以日剂量形式向无排卵女性投与组合物。医药组合物也适用于治疗和 / 或预防乳癌和 / 或作为以手术初始治疗后的辅助疗法以使复发的可能性最小化。医药组合物也适用于治疗子宫内膜（或子宫）癌和卵巢癌。

[0032] 本文中论述的所有参考文献以全文引用的方式并入本文中。

[0033] 以下实例意欲说明本发明并且不打算限制如所附权利要求书所陈述的本发明范围。

[0034] 实例 1

[0035] 制备反式克罗米酚柠檬酸盐

[0036] 克罗米酚柠檬酸盐制备如下：

[0037] 将含有过量氯化氢的 20g 1-[对 -（β - 二乙氨基乙氧基）苯基]-1,2- 二苯基乙醇于 200cc 乙醇中的混合物回流 3 小时。在真空中去除溶剂和过量氯化氢，并且将残余物溶解于乙酸乙酯和二氯甲烷的混合物中。获得在 148℃到 157℃下熔化的 1-[对 -（β - 二乙氨基乙氧基）苯基]-1,2- 二苯乙烯盐酸盐。在回流下在无水氯仿中用 N- 氯丁二酰亚胺处理此盐酸盐。将随后获得的产物转化为游离碱且用柠檬酸处理。获得在 116.5℃到 118℃下熔化的 1-[对 -（β - 二乙氨基乙氧基）苯基]-1,2- 二苯基氯乙烯的二氢柠檬酸盐。由此方法获得的克罗米酚柠檬酸盐包含介于 30％与 50％之间的顺式异构体和介于 70％与 50％之间的反式异构体。

[0038] 随后使用美国专利第 3,848,030 号的实例 31 和 32 中描述的方法从克罗米酚异构体 的外消旋混合物中分离反式克罗米酚。

[0039] 实例 2

[0040] 粒度分析

[0041] 使用被适配成用于测量当量球体积直径的仪器，如马尔文尺寸分析 2000(Malvern Mastersizer 2000) 激光衍射粒度分析器或等效仪器对反式克罗米酚进行粒度表征。在进行粒度表征之后，随后在必要时研磨反式克罗米酚，优选地在磨机旋转速率和进料速率的合适条件下使用针磨机以使粒度值处于根据本发明的上述限度内。通过使用马尔文尺寸分析 2000 激光衍射粒度分析器取样来检查研磨效率并且以类似方式检查最终粒度。

[0042] 处于根据本发明的上述限度内的微粒形式的反式克罗米酚可随后视需要与赋形剂或载剂混合并例如用于填充胶囊。由于在研磨之前或之后的粒子形状不规则，有必要通过测量与理论球形粒子具有的样品属性相关的粒子属性对其进行表征。因此为粒子分配“当量球径”。

[0043] 可以用频率对直径标绘由表征大量“未知”粒子发现的值。这给出表示样品的粒度分布的特征曲线，即尺寸分布曲线下累积百分比。可以直接读出来自于此的值或在对数概率纸上标绘以得到适当直线。平均当量球体积直径是 50％尺寸下的值。发现的平均当量球体积直径因此是具有与“未知”粒子相同属性的理论粒子的统计表示。以下是通过举例的描述。

[0044] 分析来自实例 1 的反式克罗米酚柠檬酸盐的粒度。使用如麦克龙 W.C.(McCrone W.C.) 等人，偏光显微法 (Polarized Light Microscopy)，麦克龙研究所 (McCrone Research Institute)，芝加哥 (Chicago)，1984，第 126-127 页中所述的贝克线法 (Becke line method) 用显微镜测量反式克罗米酚柠檬酸盐的折射率。使用此方法估计样品折射率是 1.62。

[0045] 异构烷油 G™(Isopar G™) 中的 0.1％ (w/v) 卵磷脂、己烷中的 0.1％ (w/v) 斯潘™ 85(Span™ 85)(脱水山梨糖醇三油酸酯) 和水中的 0.01％ (w/v) 吐温® 20(Tween® 20)(聚山梨醇酯 20) 作为反式克罗米酚柠檬酸盐的分散剂进行评估。样品容易地悬浮于两种有机分散剂中的每一个并且从悬浮液缓慢沉降。在悬浮于水性分散剂的样品中观测到部分溶解，因此水中的 0.01％ (w/v) 吐温® 20 不合适。用显微镜检查分散于基于异构烷油 G™ 和基于己烷的分散剂中的样品。样品组成和形态类似，展现长度介于 10 与 150μm 之间的晶状叶片和晶针，其聚结中的差异较小，并且观测到一些大于 400μm 的柔软聚结物。选择己烷中的 0.1％ (w/v) 斯潘™ 85 用于进一步分 析。

[0046] 样品吸收或折射率的无功分量是样品所吸收的光量的量度并且是从测量的散射模式计算粒度分布的重要参数。无法以实验方式测量样品吸收，因此其必须使用“尝试错误法 (trial and error)”使用针对特定化合物的散射模式来估计。使用以下参数收集初始粒度测量值并且使用各种样品吸收值从散射数据计算粒度分布：

[0047]

折射率	1.62
默认粒子吸收	0.01
样品测量时间	10 秒
背景测量时间	20 秒
默认泵速	1000rpm
再循环时间	60 秒
模式	通用
灵敏度	正常
粒子形状	不规则

[0048] 加权残数是所测量数据与允许将所述数据转化为粒度分布的数学模型之间的拟合优度的量度。0.01 的样品吸收指数产生最佳拟合且被选择用于所有后续粒度分析。

[0049] 在 1000rpm 的泵速下在经大致五分钟时程再循环的同时收集分散于己烷中的 0.1％ (w/v) 斯潘™ 85 中的样品的重复粒度测量值。相对于再循环时间标绘粒度 (d10、d50、d90)。d10 和 d50 二者的值在持续时间中处于狭窄范围内。d90 的减小主要出现在第一个 90 秒，此后逐渐减小。d90 的此减小表明聚结物在 90 秒内分散且在较长再循环的情况下可能出现一些消耗，因此选择 90 秒的再循环时间用于进一步分析。

[0050] 在增加泵速的情况下在持续 90 秒再循环的同时收集样品的重复粒度测量值以检查泵速对粒度的影响。d10 和 d50 的差异较小，但 d90 显示在 1500rpm 的泵速下的粒度增加，但在 2000rpm 下无进一步增加。选择 1500rpm 的泵速以使聚结物的分散和较大粒子的悬浮二者最优化并且使叶片和针的消耗最小化。

[0051] 在粒度测量后以 1500rpm 的泵速从分散池回收悬浮样品并且用显微镜检查。一级粒子相当良好地分散并且显微照片与在再循环之前收集的那些一致，并且类似数量和尺寸的叶片和针表明消耗已最小化。

[0052] 通过使用最终方法条件进行 5 次重复测量来评估方法的可重复性。d10、d50 和 d90 的相对标准差分别是 2.60％、3.42％和 10.70％。所有均处于对 d10、d50 和 d90 分别 ≤ 30％、≤ 10％和≤ 15％的 USP 推荐内。

[0053] 使用最终方法条件收集反式克罗米酚柠檬酸盐的每一个样品的一个粒度测量值。批次中的三个共用类似双峰粒度分布，而一个批次含有由大得多的粒子组成的第三模式。剩余批次是双峰但反映比具有双峰分布的较早三批的分布大得多的粒度。

[0054] 使用最终方法条件在粒度测量后收集分散于己烷中的 0.1％ (w/v) 斯潘™ 85 中的每一批反式克罗米酚柠檬酸盐的显微照片。结果与粒度结果一致。

[0055] 共用类似粒度分布的三个批次也共用主要由长度为 10-150μm 的叶片和针和一些≤ 10μm 的等径粒子组成的类似形态。测定粒度的最终条件是：

[0056]

样品折射率	1.62
样品吸收	0.01
分散剂	己烷中的 0.1％ (w/v) 斯潘 85
分散剂折射率	1.39
样品测量时间	10 秒
背景测量时间	20 秒
泵速	1500rpm
再循环时间	90 秒
模式	通用
灵敏度	正常
粒子形状	不规则

[0057] 使用莱卡 (Leica)DM LP 显微镜进行折射率测定。使用单一镜台下偏光器来观看样品。将样品放置在玻璃载片上，在样品上放置盖玻片，并且添加一滴认证的嘉吉 (Cargill) 折射率油。在使样品散焦时观测贝克线的移动。

[0058] 使用配备有聚光观察 (Spot Insight) 彩色相机的莱卡 DM LP 显微镜进行偏光显微法。在一级红色补偿器的情况下使用交叉偏光。使用 10×、20× 或 40× 物镜来观看样品。使用高级聚光 (Spot Advanced) 软件 (版本 4.5.9) 在环境温度下获得影像。

[0059] 使用配备有海德鲁(Hydro)2000μP分散单元的马尔文仪器(Malvern Instruments)MS2000获得粒度数据。使用基于体积的测量使用粒度分析2000(Mastersizer 2000)版本5.60软件收集和分析数据。使用NIST可跟踪玻璃珠作为参考标准物。

[0060] 使用最终方法条件的反式克罗米酚柠檬酸盐的粒度再现于下文中：

[0061]

批号	d10(μm)[a]	d50(μm)[b]	d90(μm)[c]
31249	4.850	13.455	76.891
16204	8.058	106.743	318.464
24712	4.038	13.902	218.573
32305	3.373	9.664	44.995
24867	4.794	13.418	70.289

[0062]

[0063] a. 总体积的10％的粒子小于指定粒度

[0064] b. 总体积的50％的粒子小于指定粒度

[0065] c. 总体积的90％的粒子小于指定粒度

[0066] 来自批号32305的反式克罗米酚的粒度分布再现于下文中：

[0067]

粒度（μm）	其下的体积%	粒度（μm）	其下的体积%	粒度（μm）	其下的体积%	粒度（μm）	其下的体积%	粒度（μm）	其下的体积%
0.010	0.00	0.105	0.00	1.096	0.10	11.482	57.87	120.226	99.04
0.011	0.00	0.120	0.00	1.259	0.28	13.183	63.80	138.038	99.72
0.013	0.00	0.138	0.00	1.445	0.60	15.136	69.20	158.489	99.97
0.015	0.00	0.158	0.00	1.660	1.12	17.378	73.95	181.970	100.00
0.017	0.00	0.182	0.00	1.905	1.93	19.953	77.98	208.930	100.00
0.020	0.00	0.209	0.00	2.188	3.10	22.909	81.29	239.883	100.00
0.023	0.00	0.240	0.00	2.512	4.72	26.303	83.92	275.423	100.00
0.026	0.00	0.275	0.00	2.884	6.86	30.200	85.99	316.228	100.00
0.030	0.00	0.316	0.00	3.311	9.59	34.674	87.62	363.078	100.00
0.035	0.00	0.363	0.00	3.802	12.94	39.811	88.95	416.869	100.00
0.040	0.00	0.417	0.00	4.385	16.94	45.709	90.13	478.630	100.00
0.046	0.00	0.479	0.00	5.012	21.58	52.481	91.28	549.541	100.00
0.052	0.00	0.550	0.00	5.754	26.82	60.256	92.50	630.957	100.00
0.060	0.00	0.631	0.00	6.607	32.58	69.183	93.84	724.438	100.00
0.069	0.00	0.724	0.00	7.586	38.74	79.433	95.28	831.764	100.00
0.079	0.00	0.832	0.00	8.710	45.14	91.201	96.72	954.993	100.00
0.091	0.00	0.965	0.02	10.00	51.59	104.713	98.02	1096.478	100.00

[0068] 相比于具有处于指定范围外部的粒度分布的反式克罗米酚，预期具有本发明的粒度分布的反式克罗米酚提供一致和改进的体内吸收／生物可用性概况。除确保一致地向胃肠道传递反式克罗米酚和从胃肠道吸收反式克罗米酚以外，指定粒度分布在制造方法期间提供较好控制。控制粒度也使带来所要粒化所需的水量的变化最小化。

[0069] 一股将以方便的调配方式单独或与另一种活性剂组合投与具有本发明的粒度分布的反式克罗米酚。以下调配物仅是说明性的并且不打算限制本发明的范围。

[0070] 实例3

[0071] 调配物

[0072] 使用以下各者制备包含反式克罗米酚的明胶胶囊：

[0073]

组分	量（毫克 / 胶囊）
反式克罗米酚柠檬酸盐	5.0-100
微晶纤维素	0-343.2
硬脂酸镁	0-8-

[0074] 将晶体形式的反式克罗米酚与 1/3 的总微晶纤维素掺合且穿过网筛以确保材料的良好分布。将剩余的 2/3 的微晶纤维素随后穿过网筛并与粉末混合物掺合。随后通过合适的研磨机器（例如共研磨®（Comil®）磨机）研磨所得混合物。添加先前穿过网筛的硬脂酸镁并与所得颗粒混合。在均一性分析后，将所得混合物囊封到明胶胶囊中。优选的明胶胶囊（尺寸 3）调配物如下：

[0075]

组分	量（毫克 / 胶囊）
反式克罗米酚柠檬酸盐	12.5
微晶纤维素	85.5
硬脂酸镁	2.0
总计	100.0

[0076] 可以根据以上方法制备包含反式克罗米酚和另一活性剂（例如芳香酶抑制剂或口服睾酮）的组合胶囊：

[0077]

组分	量（毫克 / 胶囊）
反式克罗米酚柠檬酸盐	12.5
阿那曲唑 (anastrazole)	1.0-50.0
微晶纤维素	85.5
硬脂酸镁	2.0
总计	100.0

[0078]

组分	量（毫克 / 胶囊）
反式克罗米酚柠檬酸盐	12.5
十一酸睾酮	80-200mg
微晶纤维素	85.5
硬脂酸镁	2.0

[0079] 或者，可以如下制得每一个均含有 5.0 到 100mg 反式克罗米酚的片剂：

[0080]

组分	量（毫克 / 片剂）
反式克罗米酚柠檬酸盐	5.0-100
淀粉	30-60
聚乙烯吡咯烷酮	0-8
微晶纤维素	25-45
硬脂酸镁	0.1-2.0
滑石	0-3

[0081] 对于片剂调配物来说，使反式克罗米酚、淀粉和纤维素穿过网筛（例如 45 号）且彻底混合。将聚乙烯吡咯烷酮的溶液与所得粉末混合，其随后穿过网筛（例如 14 号）。将产生的颗粒在 50℃ -60℃下干燥且穿过网筛（例如 18 号）。随后将先前穿过筛（例如 6 号）的硬脂酸镁和滑石添加到颗粒中，所述颗粒在混合之后于压片机上压缩以产生片剂。

[0082] 或者，如下制得每一个均含有每 5ml 剂量介于 1.0 与 100mg 之间的反式克罗米酚的悬浮液：

[0083]

组分	量 (mg/5ml)
反式克罗米酚柠檬酸盐	1.0-100mg
羧甲基纤维素钠	50mg
糖浆	1.25mg
苯甲酸溶液	0.10ml
调味剂	适量
颜料	适量
纯化水到	5ml

[0084] 对于悬浮液来说，使反式克罗米酚穿过网筛（例如45号）并与羧甲基纤维素钠和糖浆混合以形成调匀浆料。用一些水稀释苯甲酸溶液、调味剂和颜料并且在搅拌的情况下添加。随后添加足够水以产生所需的体积。

[0085] 或者，可以如下制备栓剂：

[0086]

组分	量（毫克/栓剂）
反式克罗米酚柠檬酸盐	100-500
饱和脂肪酸甘油酯	1000-3000

[0087] 对于栓剂来说，使反式克罗米酚穿过网筛（例如60号）并悬浮于先前使用最小所需热量熔化的饱和脂肪酸甘油酯中。随后将混合物倒入至栓剂模中并使其冷却。

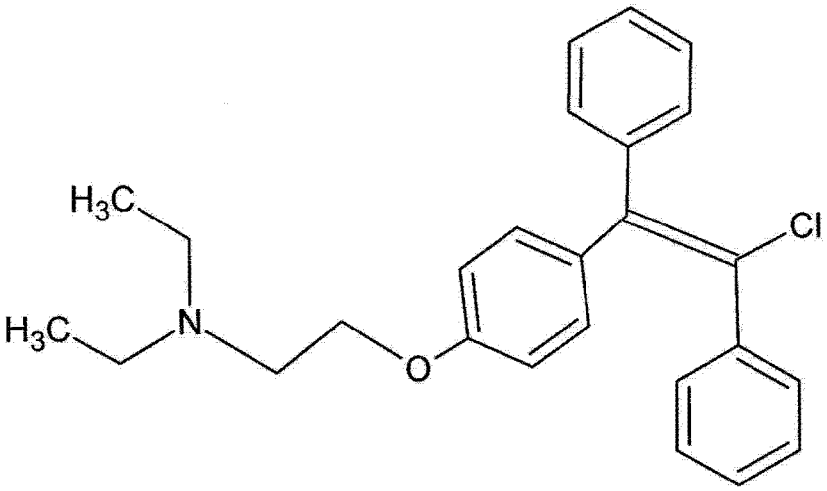


图 1