AILDENAFIL CITRATE CRYSTAL FORM O, PREPARATION METHOD AND USE THEREOF

Field of the Invention

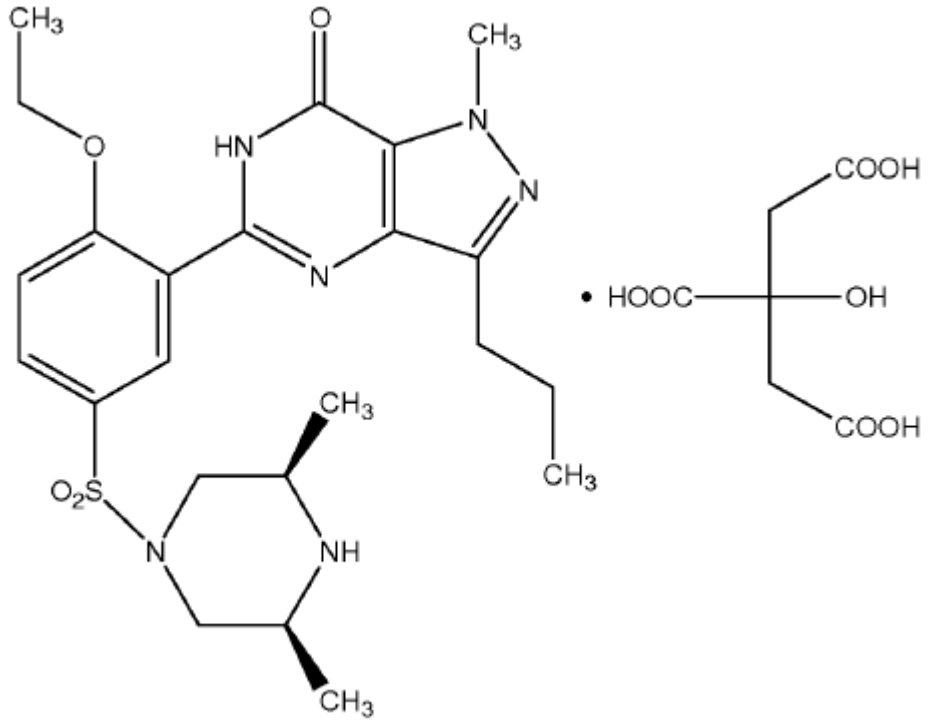
[0001] The invention relates to a crystal form O of 1-[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d] pyridine-5-group)-4-ethoxy-benzenesulfonyl]-cis-3,5-dimethylpiperazine citrate (Aildenafil citrate), and a preparation method, pharmaceutical compositions containing the crystal form O provided by the invention, and the use of the crystal form O in preparing drugs for treating Male Erectile Dysfunction (ED). This application is based on the Chinese invention patent application, of which the application date is May 10, 2010, and the application number is 201010172926.0; the contents of the above patent application are introduced into this text as a reference.

Background of the Invention

[0002] ED is a common disease, mainly defined as that, the penis cannot erect, the penis cannot ejaculate, or both; according to statistics, the incidence of ED suffered by men over the age of 40 takes 1.9%, and the incidence of ED suffered by men over the age of 65 reaches 65%. There are approximately 125 million men suffering ED in different degrees in the world; and it estimates that 322 million men may suffer ED in 2025 (Moreland RB, et al, J Pharmacol Expther, 2001, 296 (2): 225-234). Thus, it has very important clinical values and social benefits for researching and developing new safe and effective drugs or new drug delivery systems to treat ED. And for this reason, some new drugs with novel structures and unique action mechanisms are promoted to the market or are being implemented with clinical researches and preclinical studies.

[0003] The Aildenafil citrate, of which the chemical name is

1-[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d] pyridine-5-group)-4-ethoxy-benzenesulfonyl]-cis-3,5-dimethylpiperazine citrate, the molecular formula is $C_{23}H_{32}N_6O_4S \cdot C_6H_8O_7$, the molecular weight is 680.73, and the chemical structure is:



, is a new drug which is implemented with the clinical research and is effective for treating ED. The Chinese patent (of which the application number is 02100198.7) discloses the Aildenafil citrate and the preparation method thereof and the like, but does not relate to the Aildenafil citrate crystal form and the preparation method thereof.

[0004] During the process of researching and preparing the Aildenafil citrate, the invention finds that the Aildenafil citrate has multiple crystal forms; the crystal form O provided by the invention has high biological activity, high purity, good stability and excellent industrial production, is suitable for the technical process of the preparations, and is suitable for long-term storage.

Summary of the Invention

[0005] The purpose of the invention is to research an Aildenafil citrate crystal form O and a preparation method of the Aildenafil citrate crystal form O; and another purpose of the invention is to research pharmaceutical compositions containing the Aildenafil citrate crystal form O and the use of the Aildenafil citrate crystal form O in preparing drugs for treating ED.

[0006] The content of the invention is described in details with reference to the purposes of the invention.

[0007] The invention provides an Aildenafil citrate crystal form O, wherein the characteristic absorption peaks (2θ) of the Powder X-Ray Diffraction (PXRD), the D values and the relative intensities of the crystal form are as follows, and the error of the 2θ diffraction angles is +/-0.2.

Diffraction angle (2θ)	D value	Relative intensity (%)
7.600	11.6227	100
10.160	8.6992	42
13.780	6.4210	18
14.220	6.2233	32
14.980	5.9092	48
16.900	5.2419	19
18.580	4.7716	20
18.960	4.6768	32
19.300	4.5952	24
20.980	4.2308	15
22.700	3.9140	27

23.740	3.7448	27
24.700	3.6014	16
25.520	3.4875	16

[0008] The PXRD is normally used for determining the structures of the multiple crystal forms, researching the thermodynamic stability and implementing other qualitative and quantitative researches, so it is one of the most commonly-used methods for researching multiple crystal forms of the drugs.

[0009] In the invention, the precision of a light source used for measuring the 2θ value is +/-0.2^{o}, indicating that the above value allows to be taken with a certain reasonable error range, and the error range is +/-0.2^{o}; the strongest characteristic absorption peak (2θ) of the crystal form O is 7.600.

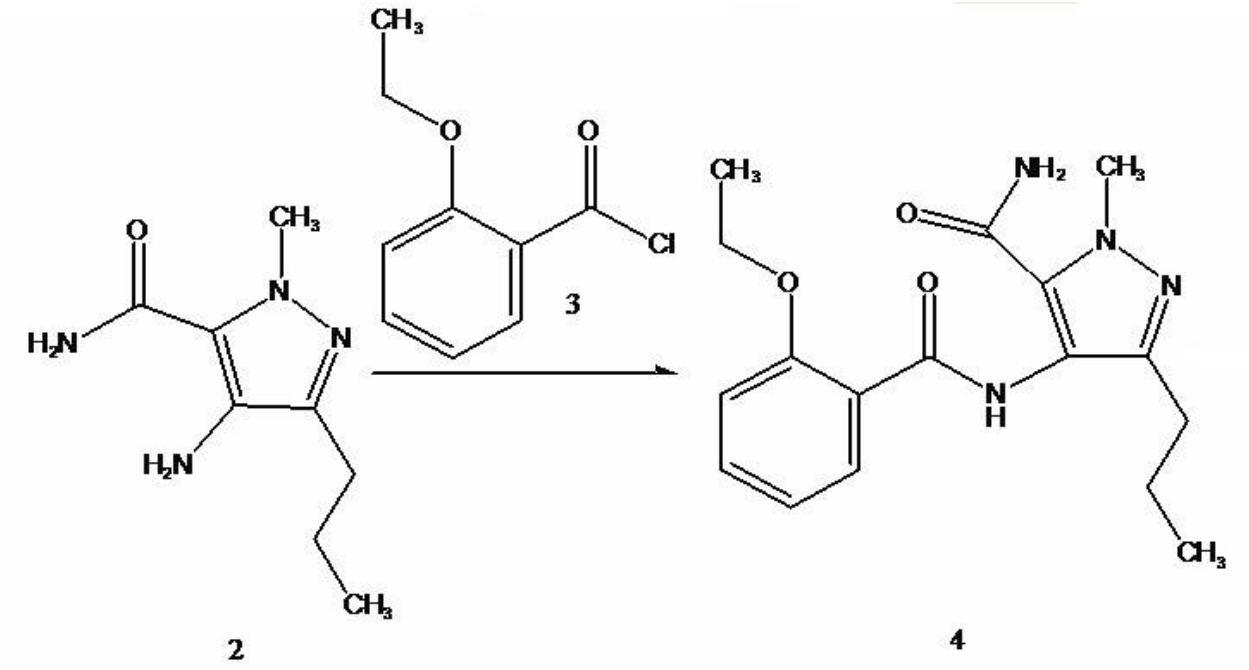
[0010] The infrared spectrogram of the crystal form O has the characteristic absorption peaks which can distinguish the crystal form O with other crystal forms in 3423+/-5cm^{-1}, 3310+/-5cm^{-1}, 3193+/-5cm^{-1}, 2980+/-5cm^{-1}, 2470+/-5cm^{-1}, 1693+/-2cm^{-1}, 1167+/-2cm^{-1}, 1023+/-2cm^{-1} and 603+/-2cm^{-1}. A KBr tablet is used when in determination, and the error range is determined according to the Chinese pharmacopoeia.

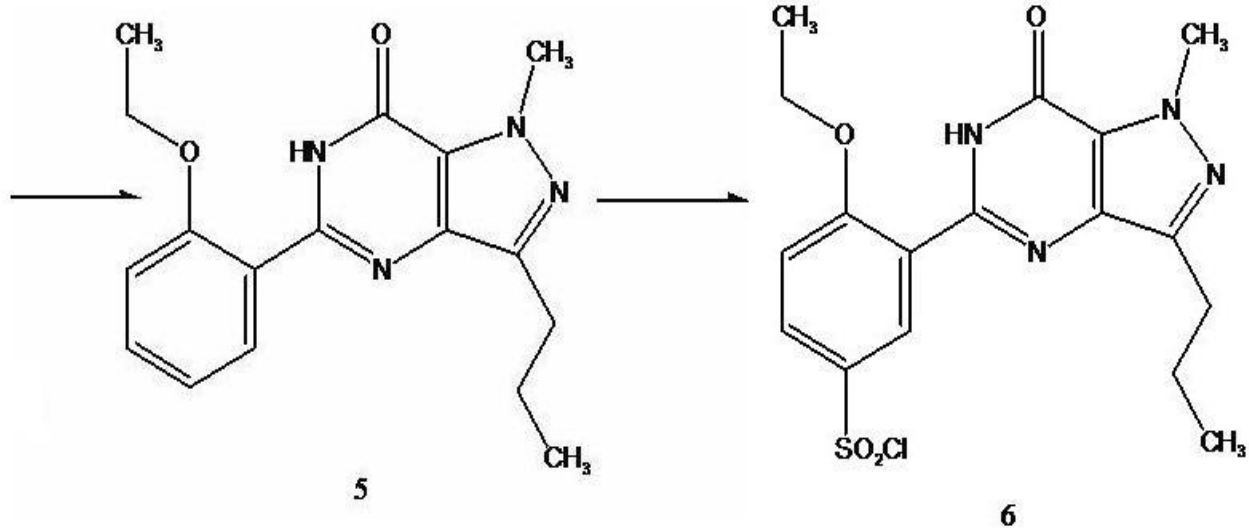
[0011] A thermal analysis result of the crystalline powder shows that the sample does not contain a crystalline solvent.

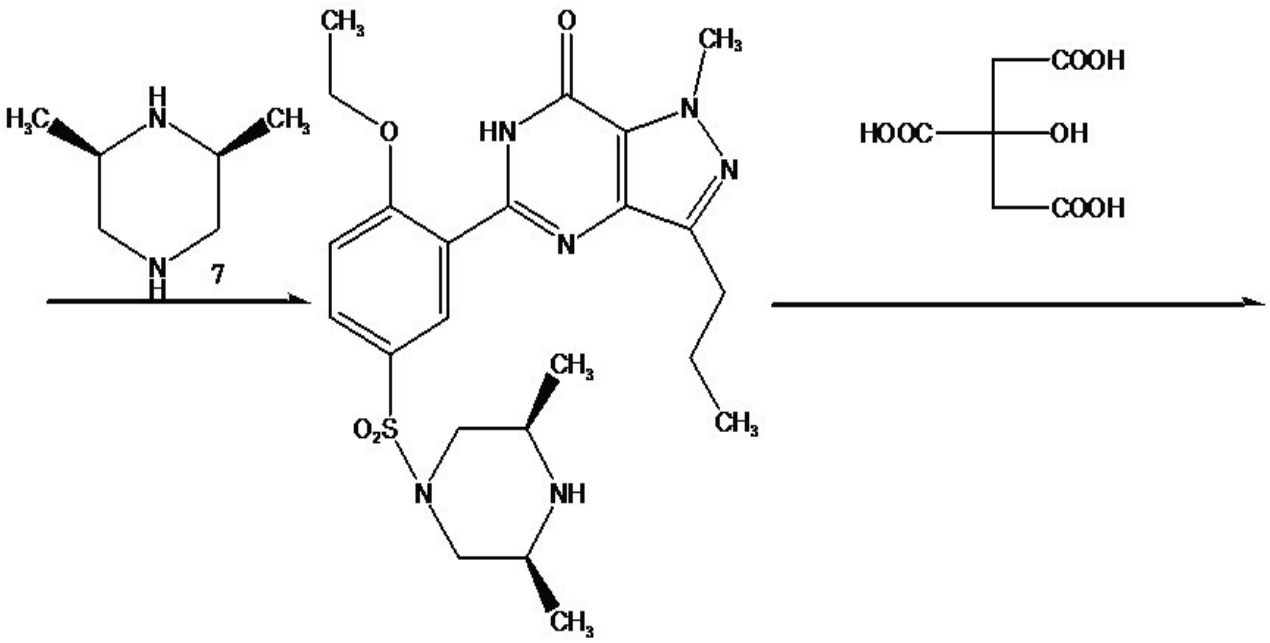
[0012] Another purpose of the invention is to disclose the preparation method of the Aildenafil citrate crystal form O, wherein, the Aildenafil citrate crystal form O can be obtained through the steps of: adding a mixture of distilled water and tetrahydrofuran which is 25 to 26 times of the amount of the Aildenafil citrate (weight-volume ratio, g/ml) into a reaction flask which is filled with the Aildenafil citrate, wherein the

tetrahydrofuran takes 5% to 15% of the volume of the mixture; stirring, heating to reach the backflow temperature, filtering the mixture after 15min to 20min while heating, cooling the filtrate to be room temperature by stirring, insulating heat and stirring for 24h to 26h, precipitating and crystallizing, filtering, indoor placing for 1h, and then moving to a vacuum drying oven to implement vacuum drying for 3h.

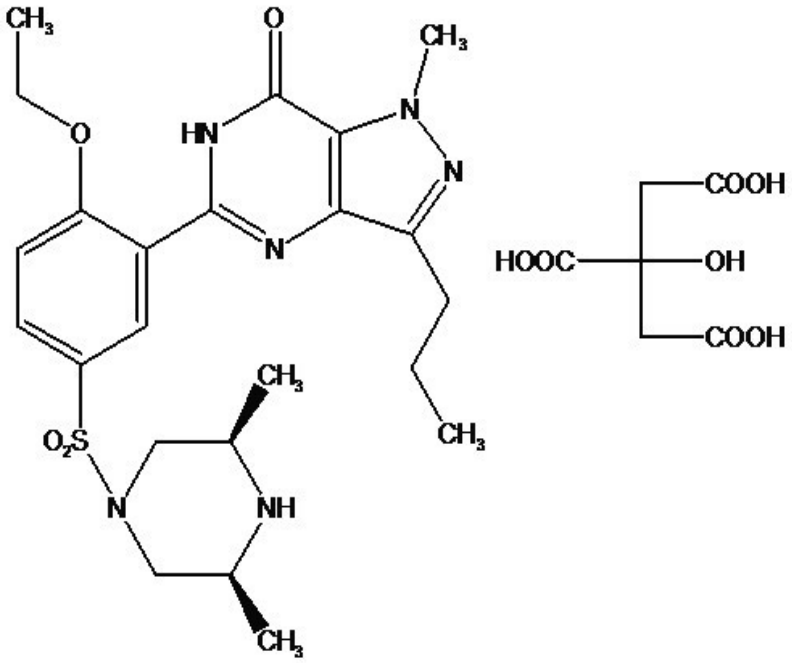
[0013] The adopted Aildenafil citrate is prepared according to the following synthetic routes:






H3C
CH3
H
N
N
H
7
CH3
O
HN
N
N
CH3
O
N
CH3
O2S
N
NH
CH3
HOOC
COOH
OH
COOH

8


CH3
O
HN
N
CH3
O
N
CH3
O2S
N
NH
CH3
HOOC
COOH
OH
COOH

1

[0014] In the reaction formula:

Compound 2: 4- amino-1- methyl-3- n-propyl pyrazol-5-formamide

Compound 3: 2- ethoxybenzoyl chloride

Compound 4: 4-(2- ethoxybenzene amide)-1-methyl-3- n-propyl pyrazol-5-amide

Compound 5: 1-methyl-3-propyl-5-[(2- ethoxy) phenyl]-1, 6-dihydro-7H- pyrazolo [4,3-d] pyridine-7-ketone

Compound 6: 1-methyl-3-propyl-5-[(2-ethoxy-5-sulfonyl) phenyl]-1,6-dihydro-7H-pyrazolo [4,3-d] pyridine-7-ketone

Compound 7: cis-2,6-dimethylpiperazine

Compound 8: Aildenafil

Compound 1: Aildenafil citrate

[0015] Wherein, the compounds 2, 3, 7 can be purchased; however, if the compounds 2, 3, 7 cannot be purchased in market, they can be prepared from easily-obtained raw materials according to a conventional synthesis method based on the document precedents; for example, the compound 2 can be prepared according to the document (Chem.Pharm.Bull.1984,32(4):1568-1577;Fine Chemicals, 2001, 18(7):396-397, and the like); the compound 3 can be prepared according to the document (Chemical Research and Application, 2002, 14(5):605-607, and the like); and the compound 7 also can be conveniently prepared according to an existing document method.

[0016] Wherein, the compounds 4, 5, 6, 8 and the Aildenafil citrate can be prepared according to the method provided by the document (US4666908: Chinese Journal of Pharmaceuticals, 2000, 31(4):145-147; Chemical Research and Application, 2002, 14(5):605-607; Journal of Shenyang Pharmaceutical University, 2002, 19(3), 174-175, and the like); when using the compound 6 to prepare the compound 8, the Aildenafil

(8) can be conveniently synthesized only by using the cis-2,6-dimethylpiperazine to replace the N-methyl piperazine. And the Aildenafil (8) can be recrystallized by methanol in order to obtain the Aildenafil citrate with higher purity. And then, the Aildenafil reacts with the equimolar citrate for 0.5 to 1h under the backflow temperature in 23 to 28 times of methanol or ethanol (weight-volume ratio, g/ml), to generate the crude product of the Aildenafil citrate; the Aildenafil citrate is used for the researching the crystal form after being recrystallized by the methanol. Confirmed by the H Nuclear Magnetic Resonance (^{1}H-NMR), C Nuclear Magnetic Resonance (^{13}C-NMR) and the like, the chemical structure of the Aildenafil citrate is proved to be correct, as shown in Fig. 3 and Fig. 4.

[0017] The above reactions for preparing the Aildenafil citrate are conventional reactions, and those skilled in the art can know that, appropriate reagents and conditions of the conventional reactions can be easily determined with reference to ordinary textbooks and related documents.

[0018] Another purpose of the invention is to provide the pharmaceutical compositions which are prepared by combining the Aildenafil citrate crystal form O and the commonly-used pharmaceutical excipients, so as to prepare various oral preparations via the conventional methods.

[0019] Any known excipient which is widely applied in this field can be used in the pharmaceutical compositions, such as a carrier, a stuffing, an expansion agent, an adhesion agent, a humidizer, a disintegrating agent, a surfactant, a lubricant or a diluent. For example, the carrier includes, but is not limited by; lactose, refined sugar, sodium chloride, glucose, starch, calcium carbonate, crystalline cellulose and silicic acid. The adhesion agent includes, but is not limited by: water, ethanol, propyl alcohol, glucose solution, starch solution, gelatin solution, Carboxy Methylated Cellulose

(CMC), methylcellulose, potassium phosphate and polyvinylpyrrolidone. The disintegrating agent includes, but is not limited by: dry starch, sodium alginate, agar powder, sodium bicarbonate, calcium carbonate, sodium dodecyl sulfate, glycerol monostearate, starch or lactose. The humidizer includes, but is not limited by: glycerin or starch. The lubricant includes, but is mot limited by: purified talcum powder, stearate, boric acid powder and Polyethylene Glycol (PEG).

[0020] The preferred medicine delivery route of the pharmaceutical compositions provided by the invention is oral. The preparation formulation includes tablets, granules, capsules, sustained-release tablets, sustained-release micro-pellets and the like. And the tablets, granules and capsules are preferred.

[0021] The quantity of the Aildenafil citrate with this crystal form contained in the pharmaceutical composition takes 50mg to 70mg according to unit formulation.

[0022] The invention also provides the application of the Aildenafil citrate crystal form O in preparing the drugs for treating ED.

[0023] Pharmacodynamic study: determination for sexual function of castrated mice

[0024] 60 Kunming strain mouse (feeding 60 mouse with 18g to 22g weight in the same period) are randomly divided into 6 groups, each 10 mouse per group, wherein, 5 groups of mouse are implemented with bilateral orchidectomy under ether anesthesia state to have castration treatment, and the rest one group of mouse are only implemented with surgical separation, without removing the testicles, which can be taken as a sham operation control group. Each group of mouse are fed in cage, and are tested after 3d. The sham operation control group and the model control group are fed with 0.5% CMC, the castration group is fed with $2mg.kg^{-1}$, $6mg.kg^{-1}$ and $20mg.kg^{-1}$ of the Aildenafil citrate crystal form O, the positive control group is fed with $6mg.kg^{-1}$ of Sildenafil; all are ig delivery modes, and the dose volumes are all

10ml.kg^{-1}. Putting the single male mice in the cage 60min after feeding the drug, and adding one female mice in each cage, recording the time that the male mice firstly catches the female mice since the female mice is put in the cage (namely, the capture incubation period), and recording the back-climbing times of the male mice within 30min. The results show that, 15mg/kg^{-1} can make the capture incubation period of the castrated mice be shortened by 201%, and make the capture times be increased by 4.4 times.

[0025] Influencing factor test:

[0026] Influence in appearance

Sample	Project	0 month	0.5 month	1 month
Aildenafil citrate crystal form O	High-temperature test High-temperature test Illumination test	White crystalline powder White crystalline powder White crystalline powder	White crystalline powder White crystalline powder White crystalline powder	White crystalline powder White crystalline powder White crystalline powder

[0027] Influence in contents (High Performance Liquid Chromatography (HPLC) area normalization method)

Sample	Project	0 month (%)	0.5 month (%)	1 month (%)
Aildenafil citrate crystal form O	High-temperature test High-temperature test Illumination test	99.96 99.96 99.96	99.96 99.95 99.96	99.95 99.96 99.96

[0028] Influence in related articles

Sample	Project	0 month (%)	0.5 month (%)	1 month (%)
Aildenafil citrate compound crystal form O	High-temperature test High-temperature test Illumination test	0.04 0.04 0.04	0.04 0.04 0.04	0.04 0.05 0.04

[0029] Influence in infrared absorption spectrum

Sample	Project	0 month	0.5 month	1 month
Aildenafil citrate compound crystal form O	High-temperature test	As shown in Fig. 2	Unchanged	Unchanged
	High-temperature test	As shown in Fig. 2	Unchanged	Unchanged
	Illumination test	As shown in Fig. 2	Unchanged	Unchanged

[0030] Influence in PXRD

Sample	Project	0 month	0.5 month	1 month
Aildenafil citrate compound crystal form O	High-temperature test	As shown in Fig. 1	Unchanged	Unchanged
	High-temperature test	As shown in Fig. 1	Unchanged	Unchanged
	Illumination test	As shown in Fig. 1	Unchanged	Unchanged

[0031] The advantages of the invention are as follows:

[0032] From the above five tables, it can see that the appearance, PXRD and the infrared absorption spectrum of the Aildenafil citrate crystal form O are all unchanged in the case of high light (4500lx+/-500lx), high temperature (60+/-2°C), high humidity (Relative Humidity (RH)92.5%) from 0 to 1 month, and this shows that the crystal form is stable, without generating crystal transformation, and the original crystal form is still remained; additionally, as the related articles and the contents are unchanged, the crystal form O has excellent chemical stability, and is suitable for manufacturing and long-termly storing the drug preparations.

Brief Description of the Drawings

[0033] Fig. 1 shows a PXRD diagram of Aildenafil citrate crystal form O;

[0034] Fig. 2 shows an infrared spectrogram of the Aildenafil citrate crystal form O;

[0035] Fig. 3 shows the ^{1}H-NMR of the Aildenafil citrate; and

[0036] Fig. 4 shows the ^{13}C-NMR of the Aildenafil citrate.

Detailed Description of the Embodiments

[0037] In order to make those skilled in the art better understand the invention, the invention is further described with reference to the embodiments and drawings below. The embodiments are only used for explaining the invention, without limiting the range of the invention in any mode.

[0038] The Aildenafil citrate adopted in the invention has been descried before; and confirmed by elemental analysis, ^{1}H-NMR, ^{13}C-NMR, DEPT and High Resolution Mass Spectrometry (HRMs), the chemical structure of the Aildenafil citrate is correct, wherein, the ^{1}H-NMR and the ^{13}C-NMR are as shown in Fig. 3 and Fig. 4.

[0039] Embodiment 1

[0040] 18.8g Aildenafil citrate crystal form O can be obtained by adding 20g Aildenafil citrate, 450ml distilled water and 50ml tetrahydrofuran in a 1000ml reaction flask, stirring, heating to reach the backflow temperature, filtering while heating after 15min, cooling the filtrate to reach the room temperature while stirring, insulating heat and stirring for 25h, precipitating and crystallizing, filtering, indoor placing for 1h, and then moving to a vacuum drying oven, vacuum drying for 3h; the refining rate is 94%, the content is 99.96% via the determination of the HPLC area normalization method. The characteristics of the Aildenafil citrate crystal form O are displayed via the detection implemented by an X-ray diffraction instrument and an infrared spectrometer (as shown in Fig. 1 and Fig. 2).

[0041] Embodiment 2

[0042] 37.6 g Aildenafil citrate crystal form O can be obtained by adding 40g Aildenafil citrate, 900ml distilled water and 95ml tetrahydrofuran in a 2000ml reaction flask, stirring, heating to reach the backflow temperature, filtering while heating after

20min, cooling the filtrate to reach the room temperature while stirring, insulating heat and stirring for 25h, precipitating and crystallizing, filtering, indoor placing for 1h, and then moving to a vacuum drying oven, and vacuum drying for 4h; the refining rate is 94%, the content is 99.96% via the determination of the HPLC area normalization method. The characteristics of the Aildenafil citrate crystal form O are displayed via the detection implemented by the X-ray diffraction instrument and the infrared spectrometer (as shown in Fig. 1 and Fig. 2).

[0043] Embodiment 3

[0044] Granules containing the Aildenafil citrate crystal form O

[0045] Prescriptions: using 50g Aildenafil citrate crystal form O, 650g lactose, 100g crospovidone, 90g PEG-4000, 135g hydroxypropyl methyl cellulose and appropriate distilled water to preparing 1000 bags.

[0046] Technique: crushing the PEG-4000 together with the Aildenafil citrate crystal form O, screening through a 80-mesh sieve, uniformly mixing with other materials, and then using the distilled water to prepare flexible materials and to granulate, drying under low temperature to package as granules.

[0047] Embodiment 4

[0048] Capsules containing the Aildenafil citrate crystal form O

[0049] Prescriptions: using 60g Aildenafil citrate crystal form O, 50g starch, 40g lactose, 10g sucrose, 35g microcrystalline cellulose, appropriate 10% polyvinylpyrrolidone ethanol solution and 1g magnesium stearate to prepare 1000 capsules.

[0050] Technique: screening the Aildenafil citrate crystal form O and auxiliary materials via a 80-mesh sieve, weighing according to the prescription quantity, taking the 10% polyvinylpyrrolidone ethanol solution as the adhesion agent, and using a

16-mesh sieve to prepare proper granules, drying under 65°C, and granulating by using a 14-mesh sieve, adding the magnesium stearate to uniformly mix, measuring the granule content, calculating the filling quantity, and then filling into the capsules.

[0051] Embodiment 5

[0052] Tablets containing the Aildenafil citrate crystal form O

[0053] Prescriptions: using 70g Aildenafil citrate crystal form O, 5g microcrystalline cellulose, 140g lactose, 10g PEG-4000, 1g magnesium stearate, 14g polyvidone K30, 10g croscarmellose sodium and appropriate distilled water to prepare 1000 tablets.

[0054] Technique: crushing the PEG-4000 together with the Aildenafil citrate crystal form O, screening through the 80-mesh sieve, using distilled water to prepare the flexible material after being uniformly mixed with other materials, using the 16-mesh sieve to granulate, drying in a drying box under 40°C to 45°C, and adding the magnesium stearate into the dry granules to mix uniformly, and then tabletting.

[0055] The above is only the preferred embodiment of the invention; for those skilled in the art, changes can exist in the embodiments and the application range according to the ideas of the invention; thus, the contents of the description cannot be understood as the limitation of the invention.

Industrial Applicability

[0056] The invention relates to a 1-[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d] pyridine-5-group)-4-ethoxy-benzenesulfonyl]-cis-3,5-dimethylpiperazine citrate or Aildenafil citrate crystal form O and a preparation method thereof; and the invention also relates to the pharmaceutical compositions containing the Aildenafil citrate crystal form O and the use thereof in preparing drugs for treating ED; in this invention,

the Aildenafil citrate crystal form O can be obtained by dissolving the raw material, namely, the Aildenafil citrate in a mixture of the distilled water and the tetrahydrofuran, stirring, heating, filtering, stirring filtrate, cooling, insulating heat, crystallizing, filtering and the like. The Aildenafil citrate crystal form O can be adopted to prepare the drugs with pharmaceutical excipients, and then used for so as to treat the male sexual dysfunction diseases. The Aildenafil citrate crystal form O provided by the invention can be produced in batch, and has excellent market prospects.

What is claimed is:

1. An Aildenafil citrate crystal form O, wherein, in a powder X-ray diffraction (PXRD) determination which takes a CuKa ray as a feature, a map thereof has the following 2θ diffraction angles, D values and relative intensities; an error of the 2θ diffraction angles is +/-0.2;

Diffraction angle (2θ)	D value	Relative intensity (%)
7.600	11.6227	100
10.160	8.6992	42
13.780	6.4210	18
14.220	6.2233	32
14.980	5.9092	48
16.900	5.2419	19
18.580	4.7716	20
18.960	4.6768	32
19.300	4.5952	24
20.980	4.2308	15
22.700	3.9140	27
23.740	3.7448	27
24.700	3.6014	16
25.520	3.4875	16

2. The Aildenafil citrate crystal form O according to claim 1, wherein, the strongest characteristic absorption peak (2θ) of the crystal form O in the PXRD determination is 7.600.

3. The Aildenafil citrate crystal form O according to claim 1, wherein, in an infrared spectrogram, the characteristic absorption peaks which can distinguish the

crystal form O with other crystal forms exist in 3423+/-5cm^{-1}, 3310+/-5cm^{-1}, 3193+/-5cm^{-1}, 2980+/-5cm^{-1}, 2470+/-5cm^{-1}, 1693+/-2cm^{-1}, 1167+/-2cm^{-1}, 1023+/-2cm^{-1} and 603+/-2cm^{-1}.

4. The Aildenafil citrate crystal form O according to claim 1, wherein, a determination result of a thermal analyzer shows that a sample does not contain a crystalline solvent.

5. A preparation method of the Aildenafil citrate crystal form O according to any one of claims 1 to 4, wherein, the Aildenafil citrate crystal form O can be obtained through the steps of: dissolving the Aildenafil citrate in 25 to 26 times (weight-volume ratio, g/ml) of mixture of distilled water and tetrahydrofuran, stirring, heating to reach the backflow temperature, filtering after 15min to 20min while heating, cooling a filtrate to be the room temperature by stirring, insulating heat and stirring for 24h to 26h, precipitating and crystallizing, filtering and drying.

6. The preparation method of the Aildenafil citrate crystal form O according to claim 5, wherein, in the mixture of the distilled water and the tetrahydrofuran, the tetrahydrofuran takes 5% to 15% of the volume of the mixture.

7. A composition formed by the Aildenafil citrate crystal form O according to any one of claims 1 to 4 with one or more pharmaceutically acceptable carriers, excipients or diluents.

8. The pharmaceutical composition of the Aildenafil citrate crystal form O according to claim 7, wherein the composition is used for preparing oral preparations.

9. The pharmaceutical composition of the Aildenafil citrate crystal form O according to claim 8, wherein, the pharmaceutical composition is applied in preparing drugs for treating Male Erectile Dysfunction (ED).