ABSTRACT

We prepared a polymorphic form of valnemulin hydrogen tartrate (Form I) to overcome the instability and irritating odor of valnemulin hydrochloride that affect its use in the production and application of veterinary drugs. The physicochemical properties of Form I were characterized by scanning electron microscopy, X-ray powder diffraction, infrared spectroscopy, differential scanning calorimetry, and thermogravimetric analysis. The results showed the crystal structure and thermal properties of Form I were very different from those of a commercially available form of valnemulin hydrogen tartrate (Form II). Form I and Form II were more stable than valnemulin hydrochloride after storage under irradiation and high humidity conditions, respectively. The solubility of Form I was 2.6 times that of Form II, and Form I was selected for use in pharmaceutical kinetics experiments in vivo. Compared to valnemulin hydrochloride, after oral administration at a dose of 10 mg/kg in pigs, Form I had similar pharmaceutical kinetic behavior but a slightly higher area under the concentration–time curve from time zero to the last measurable concentration. Consequently, Form I should be suitable for the development of simple formulations and be effective in the clinical application of veterinary drugs.

Keywords: Pharmacokinetics; polymorphism; solubility; valnemulin; pigs

INTRODUCTION

Valnemulin is a semisynthetic antibiotic derivative of pleuromutilin [1-3] that has been proposed for oral administration in the treatment and prevention of swine dysentery in the European Union [4-8]. However, valnemulin is relatively unstable during storage, and only an amorphous hydrochloride salt form has been described to date. Valnemulin is one of the active ingredients in premixed feed pellet products containing valnemulin, one-quarter to one-third of the active ingredient is lost [9]. This loss of the active ingredient leads to dosage problems, affects treatment success, and increases the cost of the end product. Consequently, various studies have investigated methods for improving the stability of valnemulin in meal and pelleted feed [10-12]; However, the suggested procedures are technically very complex and lead to substantial increases in the cost of pelleted feed.
To solve this problem, valnemulin could be reacted with an organic salt that has higher crystallinity and stability than valnemulin hydrochloride. Surprisingly, crystalline salts are more acceptable than other dosage forms for oral administration to animals. Palatability of oral pharmaceuticals is crucial to treatment success. Therefore, the production of dosage forms that have improved palatability is important [13]. We previously synthesized the organic acid salt valnemulin hydrogen fumarate, which is more stable than valnemulin hydrochloride [14,15]. We have also prepared a polymorphic form of valnemulin hydrogen tartrate (Form I), which has good stability and solubility, and its crystallization method has been patented [16].

Drugs can exist in diverse polymorphic forms that have different physicochemical properties, including melting points, densities, morphologies, solubilities, and colors. This, in turn, may affect their chemical stability, bioavailability, and ability to be processed during manufacturing and/or in the final product. In extreme cases, an undesired polymorph can even be toxic. Therefore, characterization and control of the solid-state properties of a drug are important during early product development [17]. The aqueous solubility of a molecule is one of the key determinants of the success of new compounds as medicines [18].

In the present paper, we compared the physicochemical properties, including stabilities and solubilities, of Form I and a commercially available Form II [19] of valnemulin hydrogen tartrate by using scanning electron microscopy (SEM), X-ray powder diffractometry (XRD), infrared (IR) spectroscopy, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). In addition, ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) was used to study the pharmaceutical kinetics of Form I in pigs.

**MATERIALS AND METHODS**

**Materials**

Valnemulin was obtained from Dargon Chemical Co. (95.92% purity; China). Valnemulin hydrochloride and valnemulin hydrogen tartrate were supplied by Longxiang Co. Ltd. (96.30% and 97.5% purity, respectively; China). Acetone, N,N-dimethylformamide, and tartaric acid were obtained from National Chemical Co. (China). HPLC-grade acetonitrile was purchased from Fisher Scientific Co. (USA). All water used was deionized and purified to 18.2 MΩ·cm (Millipore, USA). All other reagents and chemicals employed were of analytical purity.

**Preparation of valnemulin hydrogen tartrate polymorph—Form I**

Valnemulin (8 g) was dissolved with heating in 50 mL of 60% acetone and 40% N,N-dimethylformamide at room temperature. Then, 1.6 g of tartrate acid was added, and the resulting mixture stirred until a clear solution was obtained. Afterward, the mixture was cooled to room temperature. White crystals formed and were isolated by filtration and dried. These crystals were classed as Form I.

**Characterization of the polymorphs**

**SEM**

The morphologies and surface characteristics of the polymorphs of valnemulin hydrogen tartrate were investigated by SEM. The SEM images were recorded by using SEM-FEG instrument model XL30 (Phillips, Netherlands) according to the method described by Hacene et al. [20].
XRD measurements
Powder diffractograms were recorded by using a D8 ADVANCE XD-3 diffractometer (Bruker AXS GmbH, Germany) with CuKα radiation at 40 kV and 30 mA.

IR spectroscopy
An infrared spectrum of each polymorphic form was recorded by using a Nicolet instrument (Thermo Nicolet, USA) and the potassium bromide disk method.

DSC and TGA
The thermal behavior of samples was examined by using a SETARAM LABSYS instrument model TG-DTA/DSC system (Setaram, France). The analyses were carried out with a temperature ramp from 30°C to 250°C at 10°/min according to the method described by Baraldi et al. [21].

Stability tests under irradiation and humid conditions
For irradiation determination, Form I and Form II samples were stored at room temperature under simulated daylight irradiation for 10 days with a light intensity of 4,500 ± 500 lx. For hygroscopicity determination, samples were stored at (65 ± 5)%/25°C and (78 ± 5)%/25°C conditions for 2 days. The conditions of sample treatment, UPLC analysis, and statistical analysis matched the optimized conditions previously reported [14,15].

Solubility studies
Solubility determination was performed according to the method described by Da et al. [3,22] with some modifications. An excess amount of valnemulin hydrogen tartrate polymorphs were added to 10 mL of ultrapure water at 25°C. The suspended solution was shaken for 12 h in a shaker. After attaining equilibrium, the solution was allowed to settle for 2 h. Then, the supernatant liquid was withdrawn and filtered through a 0.22 μm membrane. The filtered samples were poured into a volumetric flask and diluted to a fixed volume for UPLC analysis. All experiments were performed in triplicate. The UPLC analysis and statistical analysis matched the optimized conditions previously reported [14,15].

Pilot pharmacokinetics study in pigs
Experimental design
A pilot study in pigs was conducted to investigate the basic pharmacokinetics of Form I in plasma. Six male Duroc x cross pigs (Chengxin Bio Co., China) with body weights of 7–10 kg were administered 10 mg/mL of Form I solution of 7–10 mL volume. A single 10 mg/kg body weight dosage was selected based on the valnemulin hydrochloride description reported by Zhang et al. [23]. A loader was used for the gastric perfusion administration. Blood samples (2 mL) were withdrawn from each pig through the precaval vein into polypropylene tubes at intervals of 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration. Blood samples were centrifuged and the plasma separated from the cells. All plasma samples were stored at −20°C until analysis, which was performed within 2 weeks. The contents and methods of the animal experiments were approved by the Institutional Animal Care and Use Committee at the China Institute of Veterinary Drug Control [certificate 2017-00121].

Sample preparation
Plasma samples (0.25 mL) were transferred into 1.5 mL polypropylene tubes, and acetonitrile (1 mL) was added. Samples were mixed by a vortex for 30 sec and then centrifuged at 12,000 r/min for 10 min. The supernatant was filtered through a PVDF 0.22 μm membrane and injected into the UPLC-MS/MS system for drug concentration analysis.
UPLC-MS/MS analysis

The method employed for UPLC-MS/MS measurement has been described in detail elsewhere [14] and was used with some modifications. The mobile phase was 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.30 mL/min. The gradient elution program was as follows: 1–4 min, 20%–80% solvent B; 4–8 min, 80%–100% solvent B; and 8–9 min, 100%–20% solvent B. The instrument was operated in multiple reactions monitoring mode with the transitions of precursor and product ion pairs of m/z 565.5/263.0 and m/z 565.5/72.0, respectively. The cone voltage was set at 20 V, the dwell time was 300 sec, and the collision energies were 20 V and 45 V, respectively. Standard calibration curves for plasma were considered linear from 0.01–2 μg/mL (R² > 0.99). The limit of quantitation was 0.01 μg/mL, the inter- and intra-day coefficients of variation were < 10%, and the recoveries ranged from 85%–90%.

Pharmacokinetics analysis

The plasma concentration–time data were analyzed by a non-compartmental method provided by WINNONLIN software (version 6.1; Pharsight, USA) and according to the method previously reported by Wang et al. [24]. All pharmacokinetic parameters were calculated and expressed as mean ± SD values.

RESULTS

Characterization of the polymorphs

SEM

The SEM results for Form I and Form II (Fig. 1) showed they had different morphologies; Form I contained rod-shaped crystals, whereas Form II contained irregular crystals.

XRD measurements

The XRD patterns of Form I and Form II are shown in Fig. 2. Form I has characteristic peaks at 5.563, 6.361, 8.741, 10.560, 11.223, 11.891, 12.775, 13.486, 15.048, 16.384, 17.689, and 17.689 (2θ ± 0.2°), whereas Form II has characteristic peaks at 6.332, 9.528, 11.229, 11.617, 11.991, 12.396, 13.584, 15.524, 16.057, 17.986, 18.122, and 20.445 (2θ ± 0.2°).

IR spectra

The occurrence of hydrogen bonds in the valnemulin hydrogen tartrate polymorphs was assessed by comparing their IR spectra (Fig. 3). The IR spectra of Form I and Form II were
simply except for slight differences in the peak intensities in the functional groups and the fingerprint regions. Form II had a stronger peak than Form I in the NH group’s bending range (1,650–1,500 cm⁻¹). A similar pattern was observed in the fingerprint region between 500 and 750 cm⁻¹ for the long carbon-chain bending band of the valnemulin molecule.

**DSC and TGA studies**

DSC and TGA profiles were recorded for Form I and Form II (Fig. 4). For profiling, a temperature program of heating from 30°C to 300°C at 10°C/min was used. The DSC curve of Form I (Fig. 4A) showed the first endothermic peak at 131°C, which corresponded to its melting point. This was followed by a second broad endothermic peak at 194°C, which was typical of the thermal behavior of melting followed by melting with decomposition. Under the same conditions, Form II showed a similar pattern with peaks at 177°C and 198°C. However, the first sharp endothermic peak (177°C) was 46°C higher than that for Form I. This
was immediately followed by a broad endothermic peak at 198°C, representative of melting with decomposition.

In addition, the two TGA curves also showed similar decomposition trends (Fig. 4B). The TGA data for Form I and Form II showed a mass loss onset at around 196°C, which was consistent with their melting with decomposition peaks. The endothermic peak of Form I was detected at 131°C with a heat of 30.91 J/g while the melting with decomposition peak was detected at 194°C with an enthalpy of 49.82 J/g (Table 1). By contrast, Form II had specific energies of 46.65 J/g for the peak at 177°C and 53.35 J/g for the peak at 198°C.

**Stability tests**

To compare the stabilities of the different crystalline forms of valnemulin under irradiation, the changes in valnemulin content from before to after storage of the two polymorphic forms were recorded. Under light irradiation, about 0.6% of Form I degraded while 0.8% of Form II degraded (Table 2). After storage at 65% relative humidity and 25°C for 2 days, the water absorption values of forms I and II were 1.3% and 1.2%, respectively. After storage at 78% relative humidity and 25°C, the water absorption value of Form I was 1.4% while that of Form II was 1.3%.

**Table 1.** DSC data of Form I and Form II crystals with the corresponding enthalpy results

| Thermal events | Form I | Form II |
|----------------|--------|---------|
| Melting point  | 131    | 177     |
| Enthalpy (J/g) | 30.91  | 46.65   |
| Decomposition | 194    | 198     |
| Enthalpy (J/g) | 49.82  | 53.35   |

DSC, differential scanning calorimetry.

**Table 2.** Content Decreases under irradiation for 10 days and water adsorption results for Form I, Form II, and valnemulin hydrochloride at high humidity

| Valnemulin forms | Content decrease (%) (irradiation) | Water adsorption (%) (65%/25°C) | Water adsorption (%) (78%/25°C) | Ref. |
|-----------------|------------------------------------|---------------------------------|---------------------------------|------|
| Form I          | 0.6                                | 1.3                             | 1.4                             | This work |
| Form II         | 0.8                                | 1.2                             | 1.3                             | [14]  |
| Valnemulin hydrochloride | 9.9                              | 4.5                             | 9.7                             | [14]  |
Solubility studies
A solid drug may have differences in its solubility because of polymorphism. The thermodynamic characteristics of Form I and Form II polymorphs were evaluated by determining their solubilities in pure water. Images were taken of solutions of Form I and Form II after 8 h of stirring (Fig. 5). At that time, Form I was a clear solution, but Form II was a suspension. Form I had higher solubility (1.600 g per 100 mL) than that of Form II (0.605 g per 100 mL) (Table 3).

Pharmacokinetic studies of valnemulin hydrogen tartrate Form I in pigs
The time course of the mean (± standard deviation) concentrations of Form I in plasma from six pigs was plotted and is shown in Fig. 6. Pharmacokinetic analysis of the data was performed using non-compartmental analysis. The main pharmacokinetic parameters are summarized in Table 4.
DISCUSSION

A new form (Form I) of valnemulin hydrogen tartrate was obtained by crystallizing valnemulin with tartaric acid; its physicochemical properties and those of a commercially available form of valnemulin hydrogen tartrate (Form II) were investigated. Visual observations of the two forms indicated they had different morphologies. The XRD pattern of the novel crystals of Form I obtained in this study was completely different from that reported previously for Form II [19]. These differences indicated that forms I and II had distinct diffraction angles and intensities and were likely two polymorphs of valnemulin hydrogen tartrate.

Different hydrogen bonding modes may affect the crystals in many compounds, such as barbiturate derivatives, sulfonamides, and oxalic acid [25-27]; thus, the occurrence of hydrogen bonds in the two valnemulin hydrogen tartrate polymorphs was assessed by comparing the IR spectra of Form I and Form II. As discussed in the theoretical study of geometries of valnemulin salts included in our previous work [14], there is hydrogen bonding between the valnemulin molecule and the tartaric acid molecule as –C=O (tartaric acid) – H–N (valnemulin). This could be attributed to the looser packing of crystal Form I than that of Form II. The H-bond involved in Form I was weaker than that in Form II; therefore, Form II displays a stronger peak than that of Form I in the NH group bending range (1,650–1,500 cm⁻¹) of the IR spectra. However, the IR spectra showed that the position of the IR absorption peak of Form I was basically consistent with that of Form II, and the slight differences in the peak intensities were insufficient to clearly distinguish the forms from each other. Although the two polymorphs of valnemulin hydrogen tartrate might have different geometries, their physicochemical properties could not be characterized by IR spectroscopy.

The differences in the DSC and TGA profiles between two forms showed that their lattice energies were quite different [19,28,29]. The intermolecular forces in Form I were weaker than those in Form II. The higher melting point and specific energy of Form II showed that its molecular arrangement was spatially more stable than that of Form I. These results were consistent with those obtained in the solubility studies.

Due to different crystallization conditions: such as solvents, temperature, pressure, and pseudoseeding with a crystal, different polymorphs may be obtained and which can have different arrangements of the molecule in the crystal [26,28]. Moreover, polymorphic solids can have different chemical and physical properties and pharmaceutical effects. Therefore, to ensure high-quality drug products, it is necessary to control the creation of polymorphic forms in pharmaceutical manufacture and formulation.

In our previous work, we reported that degradation of the amorphous valnemulin hydrochloride was 9.9% [14], which was greater than that of Form I or Form II under irradiation for 10 days. Moreover, after storage at 65% and 78% relative humidity and 25°C for 2 days, the water absorption values of valnemulin hydrochloride were 4.5% and

| Valnemulin forms                              | Target animals | Dose (mg/kg) | Cmax (mg/L)   | Tmax (h) | T1/2 (h) | AUClast (h·mg / L) | Ref.   |
|-----------------------------------------------|----------------|--------------|---------------|----------|----------|-------------------|--------|
| Valnemulin hydrochloride                      | Pigs           | 10           | 0.59 ± 0.08   | 2.20 ± 0.19 | 1.98 ± 0.21 | 3.12 ± 0.39       | [23]   |
| Valnemulin hydrogen tartrate Form I           | Pigs           | 10           | 0.60 ± 0.05   | 1.92 ± 0.01 | 2.12 ± 1.5  | 4.64 ± 0.59       | This work |

AUClast, the area under the concentration–time curve from time zero to the last measurable concentration.
9.7% [14], respectively. The valnemulin hydrogen tartrate polymorphs exhibited obvious superiority; as shown in Table 2, the water absorption values of 1.3% at 65% and 1.4% at 78% humidity of Form I, and those of Form II were 1.2% and 1.3%, respectively. Although the water absorption values of Form I was slightly higher than Form II, both forms could meet the standard of slight hygroscopicity with not more than 2% of the sample, which is in accordance with the 2015 edition of Chinese Veterinary Pharmacopoeia Appendix 9103. By contrast, the water absorption values of valnemulin hydrochloride were 4.5% and 9.7% under the same conditions [14]; thus, it could meet the standard of hygroscopicity with less than 15% but not less than 2% of the sample as per the 2015 edition of Chinese Veterinary Pharmacopoeia Appendix 9103. During application investigation, a valnemulin hydrogen tartrate Form I premix was developed by directly mixing with feed. Meanwhile, a long-term stability experiment demonstrated excellent stability after a one year storage period. The decline in the content of valnemulin was 0.86%, which was less than 5% of the sample, thus meeting the criterion of the 2015 edition of Chinese Veterinary Pharmacopoeia Appendix 9103. Therefore, valnemulin hydrogen tartrate polymorphs have better chemical stability than valnemulin hydrochloride.

The solubility of a drug is an important parameter and, to some extent, determines the bioavailability of the drug [28]. In this work, the solubility of Form I was 2.6 times that of Form II. Therefore, the excellent solubility of Form I would be beneficial for oral absorption, and Form I could be deemed a promising active pharmaceutical ingredient for drug production. Consequently, we performed pharmacokinetic studies in pigs using Form I. Various pharmacokinetic and bioavailability data for valnemulin hydrochloride have been published regarding its use in broiler chickens, rats, Muscovy ducks, layer chickens, ducks, and pigs [22-24,30,31]. However, few studies have reported similar data for valnemulin hydrogen tartrate. In this work, a pilot pharmacokinetic investigation was conducted after oral administration of Form I in pigs. Samples of plasma were prepared as detailed in the Experimental section. The results showed that the mean $C_{\text{max}}$ (0.60 ± 0.05 mg/L), $T_{1/2}$ (1.92 ± 0.01 h), and $T_{\text{max}}$ (2.12 ± 1.5 h) of Form I obtained in this study were similar to those previous reported [23] for valnemulin hydrochloride ($C_{\text{max}}$, 0.59 ± 0.08 mg/L, $T_{1/2}$, 2.20 ± 0.19 h, and $T_{\text{max}}$, 1.98 ± 0.21 h) after oral administration at the same dosage in pigs. However, the area under the concentration-time curve from time zero to the last measurable concentration (AUC$_{\text{last}}$) for Form I (4.64 ± 0.59 h·mg/L) was slightly higher than that reported for valnemulin hydrochloride (3.12 ± 0.39 h·mg/L) [23], indicating that Form I could provide a favorable effect after oral administration to pigs at 10 mg/kg. The results of this pilot study indicate that Form I may be more effective than valnemulin hydrochloride in pigs. Moreover, they provide fundamental information on the therapeutic efficacy of Form I as a polymorphic veterinary drug. Due to its good stability, Form I could be measured out efficiently and reproducibly, and it could be mixed precisely with foodstuffs during preparation and storage of animal feed. This could lead to administration of correct dosages for animals and to successful treatment. In addition, there could be a considerable decrease in the cost of the end product. Consequently, Form I has excellent potential for use in the development of simple and novel formulations.

In summary, a polymorphic form of valnemulin hydrogen tartrate (Form I) was prepared by crystallization. It was characterized by performing XRD, SEM, IR spectroscopy, DSC, and TGA. Compared to Form II, Form I has a different morphology and diffraction pattern and a lower melting point. The IR spectra of both forms were similar except for slight differences in their peak intensities, which were insufficient to clearly distinguish the two
forms. Stability results showed that Form I and Form II were more stable than commercial valnemulin hydrochloride under irradiation and high humidity conditions. Form I was more soluble than Form II. In addition, pharmacokinetics studies in pigs showed that Form I has similar $C_{\text{max}}$ and $T_{\text{max}}$ values and a slightly higher AUC$_{\text{last}}$ value than valnemulin hydrochloride. In summary, the results showing that solubility of Form I is higher than that of Form II and that its stability and bioavailability is similar to those of valnemulin hydrochloride indicate that Form I has the potential for application in novel veterinary drugs. Nevertheless, to study different polymorphs of the same drug and to develop favorable clinical medications, it is necessary to investigate the polymorphic pharmacokinetics of solid dry powder after oral administration. Further studies on the pharmacokinetics of different forms of solid-state valnemulin administered to Sprague Dawley rats are in progress and the results will be reported in the near future. Regardless, the present study provides data and technological information supporting the development of pharmaceutical polymorphs that can be used to improve drug quality and administration.

REFERENCES

1. Chen L, Yang D, Pan Z, Lai L, Liu J, Fang B, Shi S. Synthesis and antimicrobial activity of the hybrid molecules between sulfonamides and active antimicrobial pleuromutilin derivative. Chem Biol Drug Des 2015;86:239-245. [PUBMED | CROSSREF]
2. Ripley PH, Zeisl E, Horkovics-Kovats S. Use of pleuromutilin derivatives for transdermal treatment of bacterial diseases. US 6852756. 2005. [CROSSREF]
3. Tang Y, Luo J, Chen X, Wang B, Shen X, Liu J. Synthesis and in vitro antibacterial activity of four novel pleuromutilin derivatives. J Chil Chem Soc 2013;58:1537-1540. [CROSSREF]
4. Jordan FT, Forrester CA, Ripley PH, Burch DG. In vitro and in vivo comparisons of valnemulin, tiamulin, tylosin, enrofloxacin, and lincosycin/spectinomycin against Mycoplasma gallisepticum. Avian Dis 1998;42:738-745. [PUBMED | CROSSREF]
5. Stipkovits L, Ripley PH, Varga J, Palfi V. Use of valnemulin in the control of Mycoplasma bovis infection under field conditions. Vet Rec 2001;148:399-402. [PUBMED | CROSSREF]
6. Stipkovits L, Ripley PH, Tenk M, Glávits R, Molnár T, Fodor L. The efficacy of valnemulin (Econor) in the control of disease caused by experimental infection of calves with Mycoplasma bovis. Res Vet Sci 2005;78:207-215. [PUBMED | CROSSREF]
7. Li BB, Shen JZ, Cao XY, Wang Y, Dai L, Huang SY, Wu CM. Mutations in 23S rRNA gene associated with decreased susceptibility to tiamulin and valnemulin in Mycoplasma gallisepticum. FEMS Microbiol Lett 2010;308:144-149. [PUBMED]
8. Long KS, Poehlsgaard J, Hansen LH, Hobbie SN, Böttger EC, Vester B. Single 23S rRNA mutations at the ribosomal peptidyl transferase centre confer resistance to valnemulin and other antibiotics in Mycobacterium smegmatis by perturbation of the drug binding pocket. Mol Microbiol 2009;71:1218-1227. [PUBMED | CROSSREF]
9. Geissler A, Machler I, Rakoczi FJ, Schote UT. Valnemulin crystalline salts with organic acids. EP 1844008B1. 2010. [PUBMED | CROSSREF]
10. Koller K, Schwarz F. Formulation of valnemulin. US 6284792. 2001. [PUBMED | CROSSREF]
11. Wieland-Berghausen S, Rakoczi F, Cron-Eckhardt BM. Microspherules containing a pleuromutilin derivative. US 20050070486A1. 2011. [PUBMED | CROSSREF]
12. Schwarz F. Valnemulin formulation. WO 2001041758A3. 2002. [PUBMED | CROSSREF]
13. Vanacker P, Amiens FR. Palatability enhancers and methods for enhancing palatability. US 2011/0124743A1. 2011. [PUBMED | CROSSREF]
14. Zhu X, Xu S, Xu Q. Preparation of valnemulin hydrogen fumarate and its enhanced stability compared with valnemulin hydrochloride. Pharm Dev Technol 2016;21:338-345.

15. Zhu X, Xu S, Xu Q, Hu H. A preparation method of valnemulin hydrogen fumarate. CN 103073464A. 2013.

16. Xu S, Zhu X, Yi W, Zhao H. A preparation method of polymorphs of valnemulin hydrogen tartrate. CN 105061273A. 2015.

17. da Silva LM, Montanari CM, Santos OM, Cazedey EC, Ângelo ML, de Araújo MB. Quality evaluation of the Finasteride polymorphic forms I and II in capsules. J Pharm Biomed Anal 2015;105:24-31.

18. Deng J, Staufenbiel S, Bodmeier R. Evaluation of a biphasic in vitro dissolution test for estimating the bioavailability of carbamazepine polymorphic forms. Eur J Pharm Sci 2017;105:64-70.

19. Ouyang JB, Wang JK, Wang YL, Yin QX, Hao HX. Determination and correlation of solubility and solution thermodynamics of valnemulin hydrogen tartrate in different pure solvents. Fluid Phase Equilib 2014;372:74.

20. Hacene YC, Singh A, Van den Mooter G. Drug loaded and ethylcellulose coated mesoporous silica for controlled drug release prepared using a pilot scale fluid bed system. Int J Pharm 2016;506:138-147.

21. Baraldi C, Tinti A, Ottani S, Gamberini MC. Characterization of polymorphic ampicillin forms. J Pharm Biomed Anal 2014;100:329-340.

22. Sun F, Fan R, Wang J, Xiong L, Shen J, Zhang S, Cao X. Pharmacokinetics of valnemulin after intravenous, intramuscular, and oral administration in layer chickens. J Vet Pharmacol Ther 2017;40:415-418.

23. Zhang Z, Zhang CY, Guo JP, Zhu LX, Luo XY, Wang R, Liu YH. Pharmacokinetics and lung tissue concentration of valnemulin in swine. J Anim Vet Adv 2011;10:415-418.

24. Wang R, Yuan LG, He LM, Zhu LX, Luo XY, Zhang CY, Yu JJ, Fang BH, Liu YH. Pharmacokinetics and bioavailability of valnemulin in broiler chickens. J Vet Pharmacol Ther 2011;34:247-251.

25. Mesley RJ, Clements RL. Infrared identification of barbiturates with particular reference to the occurrence of polymorphism. J Pharm Pharmacol 1968;20:341-347.

26. Nagai K, Ushio T, Miura H, Nakamura T, Moribe K, Yamamoto K. Four new polymorphic forms of suplatast tosilate. Int J Pharm 2014;460:83-91.

27. Yang SS, Guillory JK. Polymorphism in sulfonamides. J Pharm Sci 1972;61:26-40.

28. Palacio MA, Cuffini S, Badini R, Karlsson A, Palacios SM. Solid-state characterization of two polymorphic forms of R-albuterol sulfate. J Pharm Biomed Anal 2007;43:1531-1534.

29. Zhou M, Ao J, Liu S, Wu C, Lai A, Gao H, Zhang G. A new polymorphic form of metoprolol succinate. Pharm Dev Technol 2017;22:58-62.

30. Yuan LG, Luo XY, Zhu LX, Wang R, Liu YH. A physiologically based pharmacokinetic model for valnemulin in rats and extrapolation to pigs. J Vet Pharmacol Ther 2011;34:224-231.

31. Sun J, Yuan L, Zhu L, He L, Luo X, Wang R, Liu Y. Pharmacokinetics and bioavailability of valnemulin in Muscovy ducks (Cairina moschata). Br Poult Sci 2012;53:374-378.