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Solid Dispersions of Fenbendazole with Polymers and Succinic Acid Obtained via Methods of Mechanochemistry: Their Chemical Stability and Anthelmintic Efficiency

Salavat S. Khalikov [1,*], Ekaterina A. Khakina [1], Marat S. Khalikov [1] and Anastasiya I. Varlamova [2]

[1] Institute of Organoelement Compounds, Russian Academy of Sciences, A.N. Nesmeyanov RAS, 119334 Moscow, Russia; eka57671232@yandex.ru (E.A.K.); marat1988@ineos.ac.ru (M.S.K.)
[2] All-Russian Scientific Research Institute for Fundamental and Applied Parasitology of Animals and Plant—A Branch of the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV", 117218 Moscow, Russia; arsphoeb@mail.ru
* Correspondence: khalikov_ss@ineos.ac.ru; Tel.: +7-926-7344999

Abstract: The substance fenbendazole is included in the composition of many anthelmintic drugs, in which the "chemical stability" parameter is one of the main characteristics when obtaining permission for the use of drugs in veterinary practice. Fenbendazole is characterized by low solubility in water and therefore the content of the substance is overestimated in its preparations, which increases the cost of the drug as well as the safety risks of pharmacotherapy. The possibilities of mechanochemical modification of fenbendazole were evaluated in order to improve the solubility index. During the mechanical processing treatment of the substance in the presence of polymeric substances, solid dispersions are formed, which have increased solubility and high anthelmintic activity. The inclusion in these dispersions of the third component, which is succinic acid, did not significantly change the solubility of fenbendazole. In all these dispersions, the substance remained unchanged both during the preparation of its solid dispersions and during their storage. When fenbendazole is modified in an organic solvent medium, the substance is partially converted into oxfendazole, which is one of its metabolites. The chemical stability of fenbendazole was confirmed via HPLC/MS and NMR spectroscopy. The anthelmintic activity of these compositions was evaluated and it was found that they have a high nematicidal activity.

Keywords: fenbendazole; polymer substances; mechanochemistry; solid dispersions; chemical stability; anthelmintic activity

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1. Introduction

Helminthiases in cattle cause significant economic damage to the livestock industry of the Russian Federation. Compositions based on the substance fenbendazole are most often used to combat helminthiases in ruminants [1]. This substance has significant drawbacks, which consist of poor solubility in water and low absorption in the digestive tract of animals, which in turn are the cause of its poor bioavailability, and therefore part of the drug is excreted from the body unchanged [2].

To improve the solubility of such substances, mechanochemical modification in the presence of polymeric substances can be used [3]. So, through mechanical processing of fenbendazole with the addition of a plant extract of licorice (EL), the solid dispersion was obtained, characterized by high anthelmintic activity [4].

Among other methods for modifying fenbendazole, its co-crystallization with some sulfonic acids should be noted [5]. Unfortunately, there are no data for the change in the solubility of fenbendazole and the biological activity of the obtained co-crystals. In addition, there are no data from experiments on the synthesis of co-crystals. Therefore, an attempt was made to obtain co-crystals of fenbendazole according to the procedure presented in the

work of Myz and colleagues [6], in which the co-crystallization of betulin with adipinic acid was studied using mechanochemistry methods. Taking into account the acidic properties of succinic acid (pH = 2.7 [7]) and its biological activity [8], we attempted to obtain a product of the interaction of fenbendazole and succinic acid. Among the interaction products, co-crystals were also expected to be obtained analogously to the work in [6]. It was expected that such interaction products should have a wide spectrum of biological activity due to the effects of overlapping properties of the original components and possible synergism. So, it was of scientific and practical interest to obtain new products based on fenbendazole with succinic acid. Such studies were not found prior to our work.

Taking into consideration the positive results on the mechanical modification of the substance of fenbendazole, it was of interest to include succinic acid in the compositions of fenbendazole and polymers, and to obtain, at the same time, promising anthelmintic drugs to evaluate their physicochemical properties, in particular the chemical stability of the substance and the effectiveness of the resulting compositions.

The aim of this work was to study the possibilities of synthesizing alternative forms of fenbendazole with succinic acid, and analyze the resulting properties and anthelmintic activity.

2. Materials and Methods

2.1. Raw Materials

Fenbendazole (FBZ)—5-(phenylthio)-2-benzimidazole carbamate (99.0%) (Figure 1) was manufactured by Changzhou Yabang Pharmaceuticals Co., Ltd. (Changzhou, China).

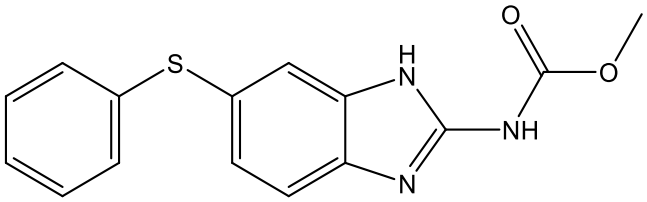

Figure 1. Molecular structure of fenbendazole (FBZ).

Succinic acid (SA) was produced by Verfarm LLC (Moscow).

Polyvinylpyrrolidone (PVP)—1 ethenylpyrrolidin-2-one brand K-30. Manufactured by Boai NKY Pharmaceuticals Ltd. (Jiaozuo, China). Batch number P160828002-0.

Arabinogalactan (AG) brand "Levitol-arabinogalactan" TU 9325-008-70692152-08. Producer—JSC "Ametis" (Blagoveshchensk, Russia).

Commercially available substances and solvents were used in the work: formic acid, acetonitrile for the co-crystallization reaction; propanol-2, dioxane-1.4, and acetonitrile (for high-performance liquid chromatography (99.9+%)); and dimethyl sulfoxide-d6 (atomic fraction D 99.8%), gaseous nitrogen and liquid, acetic acid, sodium acetate, and deionized water, which was obtained using a Sartorius Arium® mini plus laboratory water treatment system (Biohit).

2.2. Mechanochemical Modification of Fenbendazole Substance

Mechanochemical processing for obtaining two-component solid dispersions of fenbendazole (FBZ) with polymeric substances was carried out under the conditions previously described in the work in [9]. To obtain three-component solid dispersions of FBZ with succinic acid (SA) and polymeric substances, a mixture of 10.0 g of FBZ, 10.0 g of SA, and 30.0 g of a polymeric substance (polyvinylpyrrolidone (PVP) or arabinogalactan (AG)) was loaded into the drum of a roller mill LE-101. Calculated amounts of steel grinding balls (diameter 25 mm, 54 g) were added to the drum (the volume of initial substances and balls is approximately 60–65% of the vessel) and co-grinding was carried out under the following conditions: ratio of mass of starting materials to mass of balls for treatment 1:16, drum rotation speed 60–70 rpm, and processing time from 1 to 5 h with sampling for dissolution

analysis. The corresponding solid dispersions of the composition 1:1:3 were obtained in the form of free-flowing powders.

2.3. Mechanochemical Interaction of Substance of FBZ with SA

The interaction of FBZ with SA was carried out according to the procedure in [6] with the treatment in a planetary centrifugal mill replaced by mechanical treatment in an agate mortar, in which a mixture of 460.0 mg of FBZ and 460.0 mg of SA was ground for 10 min. Then, 10 mL of dioxane was added to the contents of the mortar and treatment was continued for another 5 min. The resulting mixture was transferred to a 50 mL flask and heated while stirring on a Heidolph MP 3001 K magnetic stirrer. After 5 min of heating, the white suspension turned into a transparent light-pink solution, which was left in a closed flask, in which 0.78 g of a fine light-yellow precipitate was obtained during the day (product I). Similarly, product II (using acetonitrile instead of dioxane) and product III (using propanol-2 instead of dioxane) were obtained.

2.4. Analysis of Products after Mechanochemical Modification of FBZ

The solubility of the resulting solid dispersions was determined by the amount of FBZ in the filtrate after stirring a sample of the solid dispersion in water for 3 h, using high-performance liquid chromatography (HPLC) on an Agilent 1200 chromatograph with a Zorbax Eclipse XDB-C18 column, 4.6 × 50 mm; column temperature +30 °C; diode-matrix detector at a wavelength of 290 nm. An acetonitrile acetate buffer pH 3.4 (55:45) was used as an eluent, the flow rate was 1 mL/min, and the sample volume was 5 μL [8].

Analysis of chemical stability of FBZ was performed using high-performance liquid chromatography/mass spectrometry (LC/MS) using a Shimadzu LCMS-2020 liquid chromatograph/mass spectrometer with electrospray ionization and a single-quadrupole mass detector. A Shim-pack GIST C18 3 × 150 mm, 3 μm, column with a Shim-pack GIST (G) C18 4 × 10 mm, 5 μm, pre-column was used as a stationary phase. Elution was carried out in isocratic mode with a mixture of 60 vol.% acetonitrile and 40 vol.% solution of formic acid (0.1 vol.%) in deionized water, flow rate 0.7 mL/min. The temperatures of the column thermostat, heating block, and desolvation line were 40, 400 and 250 °C, respectively. Nitrogen (99.5%, PEAK Scientific Genius XE 35 laboratory nitrogen generator) was used as a drying and nebulizing gas, and the flow rate was 15 and 1.5 L/h, respectively. The spray voltage was 4.5 kV. A portion of the analyzed samples (0.1–0.7 mg) was dissolved in 1 mL of HPLC-grade acetonitrile, and before analysis the samples were centrifuged for 2 min at 5000 rpm to precipitate the undissolved part of the sample. The sample injection volume was 2 μL. To conduct a quantitative analysis of FBZ in drug samples, the external standard method was used (initial substance of fenbendazole was used as a standard). The content of the oxidation product of FBZ, oxfendazole (5-(phenylsulfinyl)-1H-benzimilazol-2-yl)carbamic acid methyl ester) (OFZ), was determined from the ratio of signal areas of oxfendazole and fenbendazole. LabSolutions and Microsoft Excel programs were used for calculations. The stability of FBZ was determined in solid dispersions obtained in this work, as well as in samples obtained earlier (2015–2022). Changes in the composition and the appearance of the degradation products of fenbendazole were not found.

^{1}H NMR spectra were recorded on a Bruker Avance 300 spectrometer with an operating frequency of 300.15 MHz. Weighed portions of the obtained compositions (10–20 mg) were dissolved in 550 μL of dimethyl sulfoxide-D6 (SOLVEX-D). The chemical shift was determined relative to the signal shift of the residual protons of the solvent (2.5 ppm).

2.5. Anthelmintic Efficiency of Products of Mechanochemical Modification of FBZ

The study of the nematicidal activity of various forms of FBZ was carried out on a laboratory model of trichinosis on white mice experimentally infested with Trichinella spiralis at the age of 1.5–2 months at a dose of 250 larvae per animal according to the method described by us earlier [10–12].

3. Results and Discussion

3.1. The Analysis of Physical and Chemical Properties of Products, Obtained via Mechanochemical Modification of FBZ

Mechanochemical modification of poorly and insoluble anthelmintic substances from various classes of organic compounds with the help of polymeric substances makes it possible to significantly change the solubility, bioavailability, and effectiveness of drugs [4,9].

The addition of succinic acid to the compositions of the previously studied SDs of fenbendazole with PVP and AG, followed by mechanochemical treatment, made it possible to obtain the corresponding SDs with a slight increase in the solubility of FBZ. The results obtained are presented in Table 1.

Table 1. Increasing the solubility of fenbendazole (FBZ) in samples of its solid dispersions (SDs) with polymers and succinic acid (SA).

Sample and Conditions for Its Production	Sample Solubility	
	Absolute, mg/L	Increase
FBZ—initial substance	0.33	-
SD composition FBZ:PVP (1:9), 5 h m.p. *	7.9	24 **
SD composition FBZ:SA:PVP (1:1:3), 5 h m.p.	12.2	37
SD composition FBZ:AG (1:9), 5 h m.p.	7.0	21 **
SD composition FBZ:SA:AG (1:1:3), 5 h m.p.	9.6	29

*—mechanochemical processing; **—data of work [9].

In the solid dispersions of FBZ, the processes of destruction of the substance are not observed either after their preparation or during storage (5–6 years), which is confirmed by the data from the NMR and LC/MS studies (as shown in Figures 2 and 3).

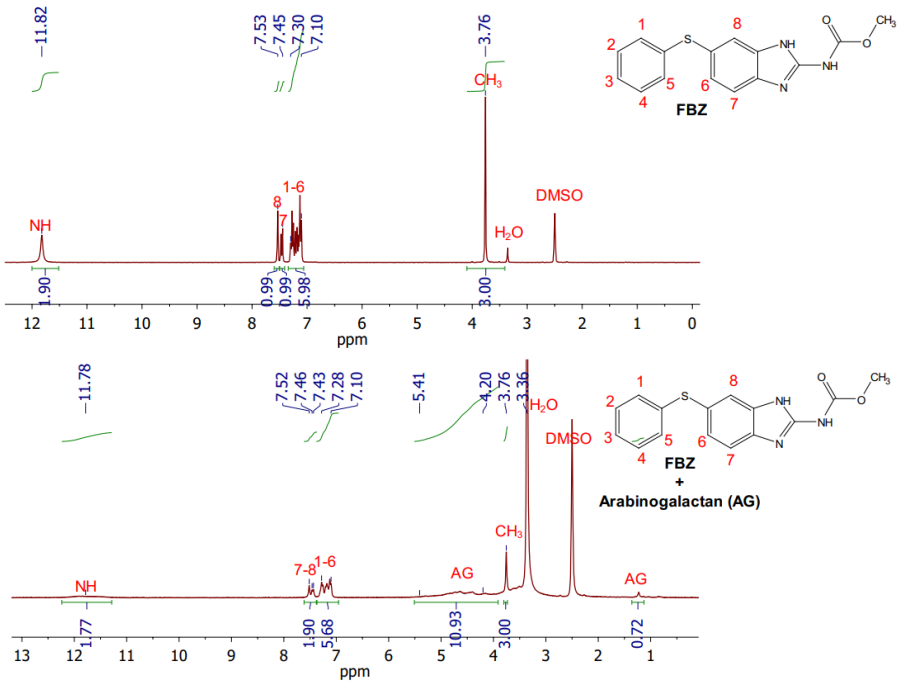

Figure 2. ^{1}H NMR spectra of SD with composition FBZ:AG (1:9) and FBZ standard (solvent—DMSO-d6).

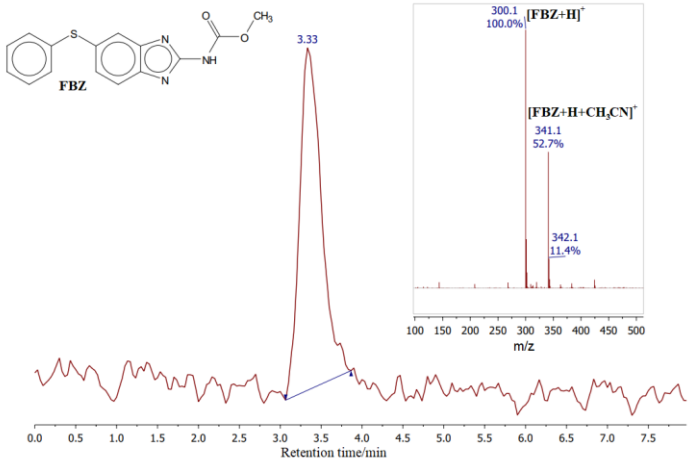

Figure 3. LC/MS of an SD with composition FBZ:AG (1:9), obtained in 2015 (total ion current chromatogram for positive ions and mass spectrum at 3.3 min).

The ^{1}H NMR spectrum of a solid dispersion (SD) with a composition of FBZ:AG (1:9) (as shown in Figure 2) contains fenbendazole signals—a broadened singlet in the region of 11.8 ppm with an integral intensity of approximately 2H, related to the proton of the NH group of the imidazole ring; a singlet at 3.75 ppm with an integral intensity of 3H, corresponding to CH_3-group protons; and a singlet at 7.5 ppm and a group of multiplets in the region of 7.0–7.5 ppm with a total integral intensity of 8H, belonging to the proton of the amide group and aromatic protons of the molecule, respectively, as well as a number of broadened signals in the range from 4 to 5.5 ppm, which can be attributed to the protons of the carbohydrate fragments of arabinogalactan. The broadening of the NH group signal at 11.8 ppm in the spectrum of the solid dispersion in comparison with the same signal in the spectrum of FBZ (Figure 2, red curve) may be due to its involvement in the process of exchange with the protons of the hydroxyl groups of the arabinogalactan polysaccharide occurring in the solution. The spectrum of arabinogalactan (Figure S1, Electronic Supplementary Information, ESI) contains only signals of water protons and residual solvent protons since it has low solubility in dimethyl sulfoxide. The appearance of additional signals in the spectrum of the solid dispersion of fenbendazole may be due to the partial fragmentation of arabinogalactan during mechanical processing and the formation of shorter molecular chains. As can be seen from Figure 2, no signals of fenbendazole destruction products (oxidated fenbendazole, for example, which has a set of aromatic proton signals downshifted compared to fenbendazole aromatic signals) are observed in the spectrum. The spectrum of arabinogalactan in deuterium oxide (Figure S2, ESI) contains broadened signals at 3.50–4.31 ppm that correspond to protons of carbohydrate fragments of AG. Unfortunately, the solubility of fenbendazole seems too low to be able to obtain its NMR spectrum in deuterium oxide. The ^{1}H NMR spectrum of SD with composition FBZ:AG (1:9) contains only signals of AG (Figures S3 and S4, ESI).

Similarly, LC/MS analysis of an SD with composition FBZ:AG (1:9) showed an absence of impurities (Figure 3).

It follows from the data in Figure 3 that the mass spectrum of 3.33 min corresponds to pure FBZ, and oxfendazole (OFZ) was not detected in the sample even with storage periods of more than 7 years.

Thus, based on the data from NMR spectroscopy and LC/MS, the stability of the substance FBZ in its solid dispersions with AG and PVP was confirmed (see LC/MS data for PVP-containing SDs in ESI, Figures S7–S10).

Taking into account the biological activity of SA in stimulating the growth of animals and increasing the resistance of their organisms [9], as well as the need to modify the previously obtained SDs of fenbendazole with polymers, we conducted studies on the inclusion of SA in these dispersions. For this, a pre-prepared physical mixture of 10.0 g

of FBZ, 10.0 g of SA, and 30.0 g of polymer (respectively, PVP or AG) was loaded into the metal drum of a roller mill, which was subjected to mechanical processing under the following conditions—modulus 1:16, rotation speed drum 60–70 rpm, and processing time 5 h. The resulting SDs of the compositions FBZ:SA:PVP (1:1:3) and FBZ:SA:AG (1:1:3) had an increased (29–37 times) solubility and, therefore, it was of interest to study their physicochemical properties and anthelmintic activity.

The analysis of the SD composition via LC/MS and ^{1}H NMR methods confirmed the stability of the FBZ substance during mechanical treatment with SA (as shown in Figures 4 and 5). The mass spectrum of negative ions of SD with composition FBZ:SA:AG (1:1:3) contains two intensive signal with m/z values of 117.2 and 257.1. These signals can be assigned to $[SA\text{-}H]^-$ and $[2SA\text{-}H+Na]^-$ ions with calculated m/z values of 117.0 and 257.0, respectively.

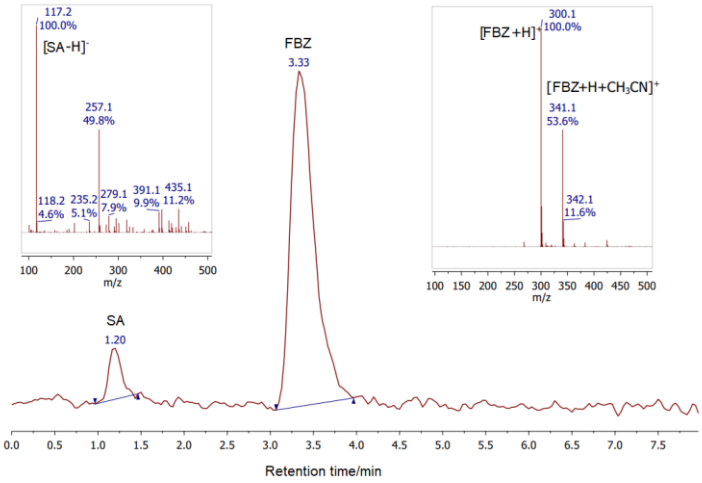

Figure 4. LC/MS of the SD of the composition FBZ:SA:AG (1:1:3) (total ion current chromatogram for positive ions, mass spectrum at 1.2 and 3.3 min).

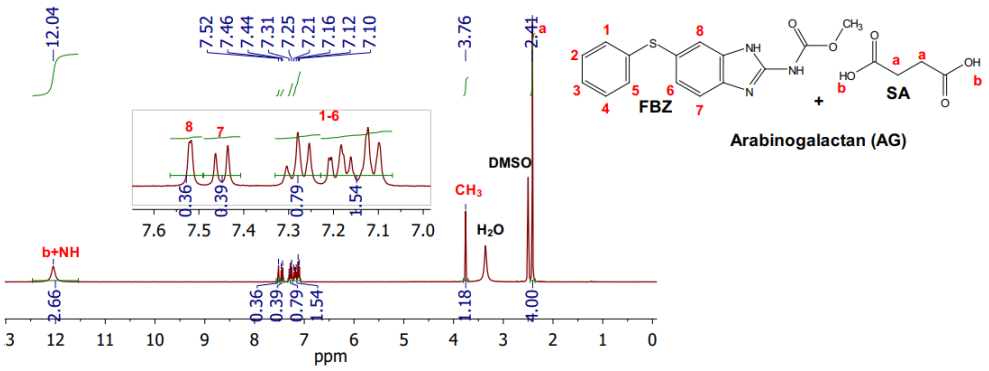

Figure 5. ^{1}H NMR spectrum of SD with composition FBZ:SA:AG (1:1:3) (solvent—DMSO-d6). Data analysis of Figures 4 and 5 confirms the stability of FBZ in these SDs. Thus, the mass spectra of both samples at 3.33 min correspond to pure FBZ, and its degradation product (OFZ) was not detected in the samples. In the ^{1}H NMR spectrum of SD of the FBZ:SA:AG composition, only FBZ and SA signals are observed; no signals of OFZ or other products of its degradation were found. The ratio of the integral intensities of the signals of succinic acid and fenbendazole indicates their molar ratio of 1:0.39, which corresponds to 1:1 by weight. It is also worth noting that ^{1}H NMR spectrum of SD with composition FBZ:SA:AG (1:1:3) compares to ^{1}H NMR spectrum SD with composition FBZ:AG (1:9). AG has low solubility in DMSO and can be salted out from solution in presence of FBZ and SA.

3.2. Analysis of Products of Mechanochemical Modification of FBZ with SA

The HPLC/MS analysis of product I showed that it is a mixture of 22% FBZ, 16% OFZ, about 7% unidentified impurities, and the rest is succinic acid (Figure 6). At the same time,

the addition product of FBZ and SA was not found, which is also additionally confirmed by the ^{1}H NMR data (Figure 7), where we can see a superposition of the spectra of the FBZ standard and SA, and the spectra also contain additional signals in the aromatic region (7.0–7.8 ppm), overlapping with the FBZ signals and related to OFZ. Product II consisted of 95% FBZ, 3% OFZ, and the rest was succinic acid, but the adhesion product was not found (Figures S5 and S11). Product III consisted of 75% FBZ and 3% OFZ, with the rest being succinic acid, and the adhesion product was not found either (Figures S6 and S12).

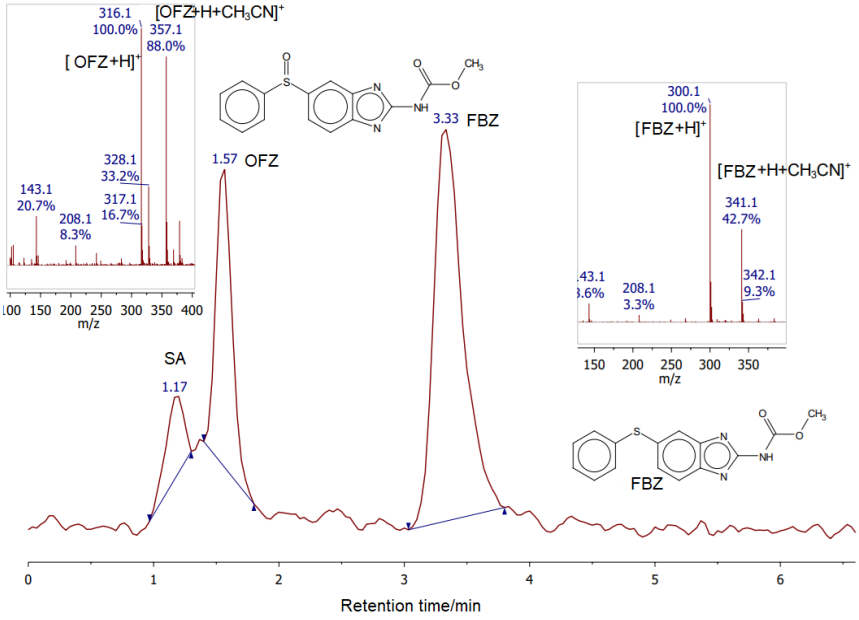

Figure 6. LC/MS spectrum of the FBZ and SA reaction product in dioxane (total ion current chromatogram for positive ions, mass spectrum at 1.57 and 3.3 min).

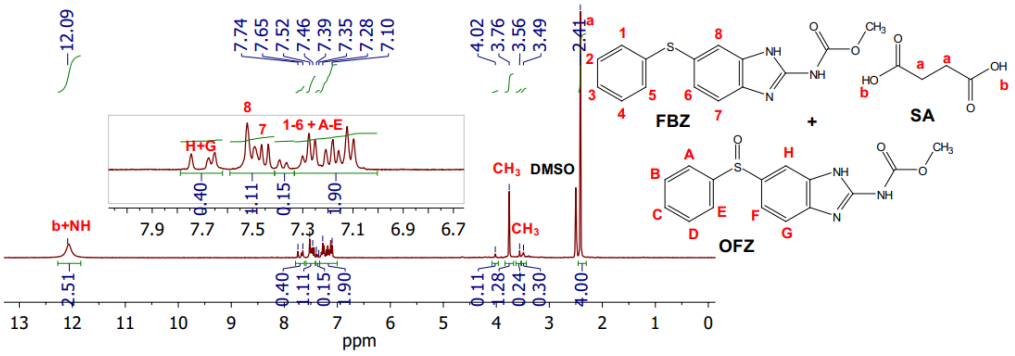

Figure 7. ^{1}H NMR spectrum of the FBZ and succinic acid reaction product in dioxane (solvent DSMO-d6).

A number of sulfides, including albendazole (ABZ) and FBZ, can be converted into their corresponding sulfoxides in a mixture of MeOH and H_2O (v/v = 2/1) under the action of light; namely, singlet oxygen plays an important role in the photosulfoxidation of sulfides [13]. It should be noted that, in our earlier studies [4,9,14], no formation of the corresponding sulfones and sulfoxides was observed in the preparation of SDs based on ABZ and FBZ with water-soluble polymers via mechanochemical methods, which was confirmed via an HPLC analysis of the products of mechanical processing. At the same time, it is known that the high anthelmintic activity of FBZ was due to the formation of primary metabolites, in particular, sulfoxide and sulfone of FBZ, which were found in the blood and milk of treated animals [15].

We discovered the formation of FBZ oxide (OFZ) when trying to modify FBZ in order to obtain its co-crystals with SA using the method in [6], in which the mechanochemical

interaction of betulin with adipinic acid was carried out in the presence of traces of a solvent, followed by boiling the resulting mass in an appropriate solvent. Myz and her colleagues used a planetary centrifugal mill "AGO-2" for the formation of the target product, and a co-crystal of betulin with adipinic acid was observed [6]. In our experiment, during mechanical treatment in an agate mortar followed by boiling in various solvents, the formation of a product of FBZ destruction was observed, but there were not even trace amounts of the product of the chemical interaction of FBZ with SA. To estimate the FBZ content in drug substances, the external standard method was used (Figures S13 and S14). The degradation of FBZ depended on the nature of the solvents (as shown in Table 2). At the same time, during the solid-phase treatment of the FBZ substance with polymers followed by storage (in the samples from 2015 and 2020), no FBZ decomposition products were observed either.

Table 2. Results of studying the chemical stability of fenbendazole in its transformation products.

Samples of Solid Dispersions, Reaction Products, Conditions, and Date of Their Receipt	The Content of Fenbendazole, %	
	Estimated	Found
FBZ:AG (1:9), mechanical processing 7 h, prod. date 27 February 2015—Composition No. 1	10	15
FBZ:PVP (1:9), mechanical processing 5 h, prod. date 30 June 2020—Composition No. 2	10	14
FBZ:SA:PVP (1:1:3), mechanical processing 5 h, prod. date 7 April 2022—Composition No. 3	20	23
FBZ:SA:AG (1:1:3), mechanical processing 5 h, prod. date 7 April 2022—Composition No. 4	20	21
Product I—product of the interaction of FBZ and SA in co-crystallization reaction in dioxane	absent	22
Product II—product of the interaction of FBZ and SA in the co-crystallization reaction in acetonitrile	absent	95
Product III—product of the interaction of FBZ and SA in the co-crystallization reaction in propanol-2	absent	75

3.3. Results of Testing of Anthelmintic Efficiancy of Products of Mechanochemical Modification of FBZ

The solid dispersion of the composition FBZ:PVP (1:9) tested on young cattle showed an efficiency of 88.4%, 97.3%, and 100,0% at doses of 2.0, 3.0, and 4.0 mg/kg body weight (BW) due to the FBZ, respectively, for nematodirosis, and 89.2%, 98.4%, and 99.5% in relation to other types of gastrointestinal strongylate, while the physical mixture of FBZ with PVP (without mechanical processing) showed 32.3% and 32.4% efficiency, and FBZ alone showed 29.7% and 27.4% efficiency at a dose of 3.0 mg/kg BW [16]. These data confirm the potential of using mechanochemical technology to create effective antiparasitic drugs.

The analysis of the results of nematicidal activity (Table 3) showed the following:

- The most active forms of application of FBZ are its two-component solid dispersions with PVP and AG (Composition 1, Composition 2);
- Three-component solid dispersions with the addition of SA (Composition 3, Composition 4) were not so active.
- Product II and product III had insufficient weak activity, despite the high content of FBZ (75% and 95%, respectively), which can be explained by the low content of OFZ (up to 3%). It is known that OFZ, as a metabolite of FBZ, has a higher anthelmintic activity [17].

Table 3. Nematicidal activity of fenbendazole and its transformation products in experimental trichinosis.

Sample	The Content of FBZ, %	Dose by Weight of Powder, mg/kg	Dose According to FBZ, mg/kg	Discovered *Trichinella spiralis*, ind./mouse	Reduction in the Average Number of Nematodes in Relation to Control, %
1.Composition No 1	10	10	1	2.5 ± 0.4	90.64
2. Composition No 2	10	10	1	0	100
3. Composition No 3	20	10	2	79.2 ± 7.1	54.17
4. Composition No 4	20	10	2	103.0 ± 8.7	40.40
6. Product II	75	-	-	120.0 ± 9.8	30.56
7. Product III	95	-	-	108.0 ± 8.5	37.50
FBZ, substance	98	2	2	132.0 ± 9.7	23.62
Control group	-	-	-	172.8 ± 12.2	-

4. Conclusions

This study of the processes of mechanochemical modification of fenbendazole showed that solid-phase modification with polymeric substances and succinic acid did not lead to chemical degradation of fenbendazole. It should be especially noted that during storage of the obtained dispersions, the destruction of the main substance, fenbendazole, did not occur. The liquid-phase modification of fenbendazole, when interacting with succinic acid in dioxane, acetonitrile, and propanol-2, led to the formation of oxfendazole, the primary metabolite of fenbendazole. The results of this study confirm the relevance of obtaining medicinal compositions using mechanochemical modification methods and studying the composition of these compositions. Thus, solid-phase treatment of the substance fenbendazole in the presence of polymeric substances leads to the formation of corresponding solid dispersions with increased solubility and high anthelmintic action. In solid dispersions, the processes of destruction of the substance are not observed either at the time of their receipt or during storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/powders2040045/s1, Figure S1: 1H NMR spectrum of AG (solvent–DMSO-d6), Figure S2: 1H NMR spectrum of AG (solvent–D2O), Figure S3: 1H NMR spectrum of FBZ (solvent–D2O), Figure S4: 1H NMR spectrum of SD with compositions FBZ:AG (solvent–D2O), Figure S5: 1H NMR spectrum of the FBZ and succinic acid reaction product II (solvent DSMO-d6), Figure S6: 1H NMR spectrum of the FBZ and succinic acid reaction product III (solvent DSMO-d6), Figure S7: HPLC of SD with composition FBZ:PVP:SA (295 nm), Figure S8: Total ion current chromatogram of SD with composition FBZ:PVP:SA, Figure S9: HPLC of SD with composition FBZ:PVP (295 nm), Figure S10: Total ion current chromatogram of SD with composition FBZ:PVP (signal at 0.867 min corresponds to PVP), Figure S11: Total ion current chromatogram of product II, Figure S12: Total ion current chromatogram of product III, Figure S13: Outer standard method calibration for FBZ quantification (295 nm), Figure S14: Outer standard method calibration for FBZ quantification (total ion current for positive ions).

Author Contributions: S.S.K.: data curation, methodology, writing—original draft and editing, conceptualization, formal analysis, visualization; E.A.K.: analysis via LC/MS and NMR methods, formal analysis, visualization; M.S.K.: investigation of the mechanochemical modification of fenbendazole, formal analysis, software; A.I.V.: study of nematocidal activity, formal analysis, writing—section on biological tests. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Study of anthelmintic activity carried out in accordance with the Guidelines for exexperimental study of new pharmacological chemical substances of substances (Khabriev R.U. Guide to experimental (preclinical) study of new pharmacological substances. M.: Medicine, 2005. 832 p. and Riviere J.E., Papich M.G. Veterinary pharmacology & therapeutics. 9th ed. Hoboken: Willey-Blackwell, 2009. 1541 p.), Rules adapted by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg,

1986), and the Rules of Good Clinical Practice of the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199n, dated 1 April 2016).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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