Research Article

Development and Evaluation of Parenteral Solution containing Florfenicol and Flunixin Meglumine for Veterinary Use

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ABSTRACT

Parenteral dosage forms are one of the most commonly used pharmaceutical dosage forms in veterinary medicine, at the same time the combination therapy of multiple drug products is a common practice in this field. The aim of this research was to develop a high quality, stable solution that combines Florfenicol and Flunixin meglumine in a multidose injectable dosage form. Different organic solvents were tested to determine the optimal solvent for the active substances in the formulation. Citric acid was used in the formulation to overcome the problem of Flunixin instability and ethyl alcohol was incorporated in the formula in order to enhance syringeability and injectability of the solution. All prepared formulations were evaluated for their chemical and physical stabilities. The candidate formula showed good physical and chemical stability after two weeks of storage at both room temperature and accelerated conditions. Samples from three pilot scale batches were stored at accelerated stability conditions for 6 months and didn't show any significant physical or chemical instability. A generic parenteral solution drug product containing Florfenicol and Flunixin meglumine for veterinary use was successfully developed using mixture of organic solvents and excipients.

Keywords: Florfenicol, Flunixin, parenteral, veterinary, formulation.

INTRODUCTION

Parenteral term is used in pharmaceutics to describe drug products administered by injection, examples for this route include intravenous (IV), intramuscular (IM), subcutaneous (SC), intracardiac and intraspinal injections. The parenteral route is one of the most effective routes for drug administration especially in animals. There are various forms of pharmaceutical products that can be administered by this route such as solutions, emulsions and suspensions.[1,2]

Veterinary parenteral dosage forms include aqueous organic solutions, oily solutions, emulsions, aqueous suspensions, oily suspensions, and sustained release implants. While, veterinary dosage forms frequently contain the same pharmaceutical ingredients that are used in human dosage forms, few of them contain active ingredients that are not used in humans and intended for animal use only. Examples of API's that have been developed specifically for animal use include some antimicrobial agents classified under sulfonamides, fluoroquinolones, macrolides, and chloramphenicol derivatives.[3]

As in human drug products, the formulation of animal preparations require fundamental knowledge in pharmaceutics, technology, dosage form design, pharmaceutical operations, quality control testing and in performing pre-formulation studies.[4]

Florfenicol and flunixin meglumine is a multidose injectable solution, which contains 300 mg florfenicol and 16.5 mg flunixin as flunixin meglumine per mL.

This drug product combination is intended to treat bovine respiratory disease (BRD) associated with *Pasteurella haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma bovis,* and to control BRD associated with pyrexia in beef and non-lactating dairy cattle.[5,6]

There are many pharmaceutical companies, that produce veterinary medicines containing the antibacterial florfenicol injectable solution [7,8] or the anti-inflammatory flunixin meglumine injectable solution[9–11], but the only product containing a combination of both APIs is *Resflor Gold*® injectable solution, the brand name is produced by Intervet/Merck Animal Health Company. [10,12]

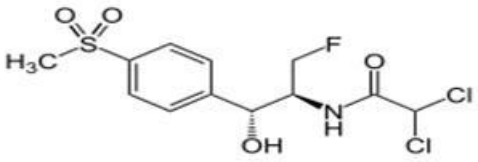
Florfenicol is a phenicol antibiotic, classified under the amphenicol group of antibiotics, which includes chloramphenicol and thiamphenicol. It is a fluorinated derivative of thiamphenicol, with the chemical name 2,2-dichloro-N-1-(fluoromethyl)-2-hydroxy-2-[(methyl sulfonyl) phenyl] ethyl]-acetamide.[13–15] The structure of florfenicol is shown in Figure 1.

Florfenicol is a white or almost white crystalline powder, practically insoluble in water, soluble in acetone and DMF, soluble in ethanol (50 mg/mL), DMSO (100 mM), water (1.32 mg/mL, pH 7) [14]

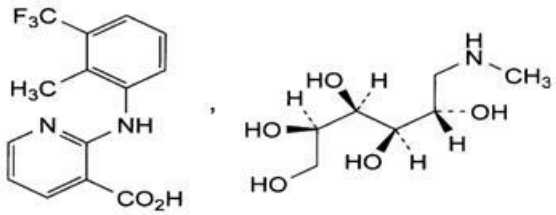
Flunixin meglumine is cyclo-oxygenase inhibitor analgesic, a non-steroidal anti-inflammatory (NSAID). It is used in animals to reduce pain and inflammation associated with serious and chronic disorders of endotoxic or septic shock and mastitis. Flunixin meglumine is 2-[[2-Methyl-3-(trifluoromethyl) phenyl] amino] pyridine-3-carboxylic

acid, 1-deoxy-1- (methylamino)-D-glucitol.[16–18] The structure of flunixin meglumine is shown in Figure 1.

Flunixin meglumine is a white or almost white, crystalline powder, freely soluble in water and in methanol, practically insoluble in acetone its pKa is 5.82,.[16,17]



Florfenicol



Flunixin meglumine

Figure 1: Chemical structure of florfenicol and flunixin meglumine

MATERIALS AND METHODS

Instrumentation

Climatic chamber KBF 240 Binder, Steam autoclave #1 STE-18L MRC Ltd., Viscometer DV1MLVTJ0 Brookfield, Dionex-Ultimate 3000 HPLC system, equipped with LPG-3400SD pump, WPS-3000SL autosampler, TCC-3000 column oven, and DAD-3000 UV–VIS diode array detector. Chromeleon Data system Software (Version 6.80 DU10A Build 2826 (171948)) was used for HPLC data processing and evaluation. The used of double distilled water was obtained from Aquatron equipment model A 4000D.

Chemicals and reagents

Active ingredients (florfenicol and flunixin meglumine) were purchased from Sigma Aldrich. Florfenicol, Flunixin meglumine, N-methyl-2-pyrrolidone, Ethyl alcohol and Glycerol formal were donated by local Pharmaceutical Company. Propylene glycol was purchased from Dow Chemical Co. Polyethylene glycol 400 was purchased from OXITENO and Citric acid was purchased from Merck. The acetonitrile used was HPLC grade and water was obtained by double distillation. Other reagents such as phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were analytical grade and purchased from Merck and Sigma Aldrich.

Pre-formulation

Drug product characterization

Before starting the experimental part, the critical quality attributes, the desired quality characteristics and properties of the finished product such as assay, sterility, stability, safety, viscosity and syringeability were all defined.

The final formulation to be developed should contain florfenicol 300 mg per mL and flunixin meglumine 27.4 mg per mL to give a 16.5 mg final concentration of flunixin in a mixture of different excipients.

Selection of excipients

Since the developed product is a parenteral solution, solubility of the active materials is critical. The use of solubilizing agents, antimicrobial preservative, acidifying agents, stabilizers, complexing agent and viscosity reducing agents were evaluated.

Depending on literature survey, characterization of the two active materials and the knowledge of their physiochemical properties, a number of excipients were selected to perform formulation and to achieve the required final chemical, physical and microbiological properties of the developed drug product.

Flunixin meglumine have a good solubility in water while florfenicol solubility is better in organic solvents such as N-methyl-2-pyrrolidone (NMP), polyethylene glycol and glycerol formal. Taking in consideration the two API's solubility, similar products containing florfenicol, NMP was determined to be the main solvent in this formulation; because it can dissolve not only florfenicol but also flunixin meglumine.

Other solvents were investigated such as water, ethanol, propylene glycol (PG), glycerol formal (GF) and polyethylene glycol 400 (PEG). NMP was the candidate solvents constituting about 80% of the formulation.

Ethyl alcohol (EA) and water were used to reduce the drug product viscosity to a suitable level that provides acceptable syringeability and injectability. Citric acid (CA) was used to stabilize Flunixin meglumine, while lactic acid (LA) was used as an acidifying agent in this water containing formulation.

Analysis

Physical tests

During the study, the appearance of the product was checked visually, and was compared with the patented product. The viscosity was measured using a Brookfield viscometer DVI, spindle type # (LV-02) # 62. The tests were carried out at two rotation speeds, 50 and 100 rpm, at 25°C.

Analytical assay development

A preliminary chromatographic analytical method for determination of Florfenicol and Flunixin was developed and validated, as a stability indicating method, to evaluate the finished product during development and stability testing. The analytical validation study of the developed method has been published as a novel scientific paper [6]

Sterility

The sterility of the developed product was tested using the membrane filtration method described in the United States Pharmacopeia (USP).

Accelerated stability study

The study was conducted in accordance with ICH guidelines regarding selection of batches, storage conditions testing frequency and acceptance criteria. Three pilot batches of the candidate formulation of the developed drug product were prepared for the study with the same strength as the product to be marketed and without any overage. A 100-mL amber glass vial (type-II) contained in a cardboard box was used as a primary packaging materials.

Sufficient samples of each batch, in their final packaging, were stored at two controlled storage conditions and samples were periodically tested according to the testing program **(Table 1)**

Table 1: Storage conditions and testing frequency

	Temperature	Humidity	Testing frequency
Accelerated conditions	40° C	75%	0, 3, and 6 months
Normal conditions	25° C	60%	

The physical, chemical and microbiological tests were performed in accordance with the testing frequency program.

RESULTS AND DISCUSSION

Formulation development

Different formulation trials were prepared to determine the final formula and the best formulation procedure as summarized in **Table 2.** These formulations were prepared according to the quantities specified in the Table using different manufacturing procedures. The product was manufactured and filled using aseptic techniques in an aseptic environment.

Table 2: Summary of formulation trials that were prepared to choose the final candidate formula and manufacturing procedure

Material	Flr	Flx	NMP	PEG	GF	LA	CA	EA	Water	PG
Unit	g	g	mL	mL	mL	mL	g	mL	mL	mL
Formula (Quantities per 100 mL)										
FF1	30.0	2.74	30.0	20.0	18.0	0.0	0.0	0.0	0.0	15.0
FF2	30.0	2.74	30.0	8.0	30.0	0.0	0.0	0.0	0.0	15.0
FF3	30.0	2.74	30.0	8.0	15.0	0.0	0.0	0.0	15.0	15.0
FF4	30.0	2.74	30.0	8.0	12.5	2.5	0.0	0.0	15.0	15.0
FF5	30.0	2.74	30.0	8.0	20.0	0.0	0.0	0.0	10.0	15.0
FF6	30.0	2.74	30.0	8.0	17.5	2.5	0.0	0.0	10.0	15.0
FF7	30.0	2.74	30.0	8.0	10.0	0.0	0.0	20.0	0.0	15.0
FF8	30.0	2.74	30.0	8.0	30.0	0.0	1.0	0.0	0.0	15.0
FF9	30.0	2.74	30.0	8.0	10.0	0.0	1.0	20.0	0.0	15.0

In formulas FF1 and FF2, Florfenicol and flunixin meglumine were dissolved completely in N-methyl-2-pyrrolidone (NMP), then while mixing continuously, a mixture of about 80% glycerol formal (GF) and polyethylene glycol 400 (PEG) was added, this was followed by the addition of the other excipient in addition to propylene glycol (PG). Finally, the total volume was accurately completed by GF. In trials FF3 to FF7 Florfenicol and flunixin meglumine were dissolved completely in NMP, then while mixing a mixture of about 80% GF and PEG was added followed by the addition of a mixture of water and PG, at the end the total volume was accurately completed by GF.

In trials FF8 and FF9 Citric acid was completely dissolved in NMP. Flunixin meglumine was then added while mixing till completely dissolved, after that florfenicol was added and mixed until completely dissolved, other excipients were added during continuous mixing. The final drug product was filled in 100-mL amber glass vials, type II as the primary packaging material, each vial was closed with rubber stopper and aluminum cap, labeled and packaged in a well-designed and elegant cardboard carton box as secondary packaging material.

The quality of the prepared trials was evaluated regarding chemical and physical properties. As a starting point, experiment FF1 was somewhat satisfactory except for the high viscosity of the solution. Modifications were necessary to decrease the viscosity, thus the amount of PEG was reduced as seen in formulation FF2, but this approach was not effective. The choice of incorporating a

viscosity decreasing agents in the formulation, such as water or ethyl alcohol, was used concurrently in experiments FF3, FF4, FF5, FF6 and FF7. A 15% of water was used in experiments FF3 and FF4 at two different pH values using lactic acid as acidifying agent, the viscosity was decreased but unfortunately, there was some turbidity in the solution after a few days of the preparation due to the precipitation of florfenicol. In experiments FF5 and FF6 the water percentage was reduced to 10%, the viscosity was good in both experiments but some precipitation occurred in experiment FF6 which had the acidic pH. Formulation FF5 showed the best viscosity compared to other formulations that contained water. In experiment FF7where ethyl alcohol was used, the viscosity of the solution was better than that obtained by FF5; therefore, FF7 formula was selected for further evaluation and testing.

Samples from experiment FF7 were analyzed and the assay of both florfenicol and flunixin were determined using the previously developed HPLC assay method, florfenicol results were acceptable but there was a significant loss in the flunixin assay. In experiment FF8, citric acid was used in the formulation to stabilize the flunixin meglumine, also it was used FF9 experiment in the presence of ethyl alcohol, and the two formulations had acceptable assay results for both florfenicol and flunixin.

Formula FF9 showed the best results regarding the viscosity of the solution and the assay of the active materials, in consequence it was selected as the candidate formula.

Formulations from FF9 were retained for a couple of weeks at room temperature for fast evaluation of stability before submitting it to the incubator for accelerated stability study.

The obtained results of analysis were excellent for both physical appearance and chemical assay tests.

Accelerated stability study

Accelerated stability study was performed on three pilot batches of the candidate formula FF9 stored for 6 months at two storage conditions and tested frequently as recommended by ICH guidelines. The tested quality parameters assayed were; physical appearance, viscosity, and sterility.

Results of the stability testing after 6 months, under different storage conditions, didn't show any significant change as seen in Table 3, also no turbidity was found suggesting that the candidate formula FF9 is physically and chemically stable. This formulation was selected as the final formulation for the developed drug product Florfenicol and Flunixin meglumine injectable solution.

Table 3: Results of the accelerated stability study

BN	Month	Assay %		Color	Clarity	Viscosity (mpa.s)	Sterility
		Flr	Flx				
Storage condition 1: 25°C/60% RH							
A	0	99.3	99.0	Light yellow	Clear	25.2	Sterile
	3	102.5	98.9	Light yellow	Clear	NA	NA
	6	99.8	98.9	Light yellow	Clear	29.4	Sterile
B	0	99.1	98.7	Light yellow	Clear	25.8	Sterile
	3	102.4	98.9	Light yellow	Clear	NA	NA
	6	99.6	99.4	Light yellow	Clear	27.5	Sterile
C	0	99.7	98.9	Light yellow	Clear	26.1	Sterile
	3	102.8	98.7	Light yellow	Clear	NA	NA
	6	100.2	101.0	Light yellow	Clear	28.7	Sterile
Storage condition 2: 40°C/75% RH							
A	0	99.3	99.0	Light yellow	Clear	25.2	Sterile
	3	102.3	100.8	Light yellow	Clear	NA	NA
	6	98.8	97.9	Light yellow	Clear	29.7	Sterile
B	0	99.1	98.7	Light yellow	Clear	25.8	Sterile
	3	102.3	101.3	Light yellow	Clear	NA	NA
	6	98.6	97.3	Light yellow	Clear	26.5	Sterile
C	0	99.7	98.9	Light yellow	Clear	26.1	Sterile
	3	101.1	102.3	Light yellow	Clear	NA	NA
	6	98.7	97.2	Light yellow	Clear	28.6	Sterile
Acceptance criteria		**±5% of initial**		**Light yellow**	**Clear**	**NA**	**Sterile**

CONCLUSION

A generic parenteral drug product containing Florfenicol and Flunixin meglumine, intended for veterinary use, was successfully developed using a mixture of organic solvents and excipients. The product showed excellent physical and chemical stability after storage under different conditions, as recommended by the official accelerated stability testing guidelines.

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