ISSN: 0975-5772
Masasoud Mahmoudian et al / Journal of Pharmaceutical Science and Technology Vol. 2 (12), 2010,432-435

Pharmacokinetics of Florfenicol in Young Male Calves using HPLC

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Abstract:
Florfenicol is a wide spectrum antibiotics and currently used against the infection of various animals**.** We determine the pharmacokinetics parameter of this drug in young male calves. Florfenicol was administered via IM route at a dose of 20mg/kg and measured using an HPLC method with UV detection and cloloramphenicol as internal standard in plasma samples. Pharmacokinetic Parameters such as $T_{1/2}$ C_{max}, T_{max}, and $AUC_{0-\infty}$ were calculated.
Keywords: *Florfenicol, pharmacokinetics, HPLC*

Introduction:

Florfenicol is a wide spectrum antibiotic and currently used against the infection of various animals, such as bacterial meningitis of calves. Florfenicol is an analog of chloramphenicol, however does not carry the risk of inducing human aplastic anemia that is associated with chloramphenicol and is effective against pathogen resistance to chloramphenicol. A major mechanism of bacterial resistant involves the presence of chloramphenicol acetyltranferase (CAT) in resistance organism. The structural modifications in the design of florfenicol, substitution of a fluorine atom for the hydroxyl group at C-3 site, prevent acetylation by CAT [2]. Consequently, florfenicol is active at lower concentration than chloramphenicol against several chloamphenicol resistant pathogens involved in common infection in domestic animal.

During last several years, different reports have been published on its pharmacokinetics in various domesticated animals. The objective of this study was to investigate its bioavailability and kinetic disposition in healthy young male calve.

Material and Methods:

Animals

12 healthy, young male calves (3-3.5 months old, weighed between 83 to 175 Kg), from Jojak animal farm (Tehran – Iran) were used. The animals were previously habituated to standing stalls and haltered to facilitate handling. All animals were examined prior to the study and only healthy calve were used. The research protocol was approved by the Animal Care Committee of our department.

Dosing and sample collection

Florfenicol injection solution containing 300mg/ml florfenicol (Razak Pharmaceurical Co. Tehran - Iran) was administered to all calves via IM rout at a dose of 20 mg/kg and blood samples were collected from right jugular vein before and after 1.0, 1.5, 2.0, 2.5, 3.0,3.5, 4.0, 6.0, 8.0, 24.0 and 48.0 hours after drug administration. The blood samples were collected in 10 ml evacuated heparinized tubes. They were centrifuged and separated plasma stored at -20°C until analysis.

HPLC assay

Plasma samples were allowed to thaw at room temperature. 250µl of IS solution (20µg/ml chloramphenicol in methanol), 1ml Ammonium phosphate buffer (pH=7) and 4ml ethyl acetate were added to 250µl plasma sample. The mixture was shaking for 2 min. and then centrifuged for 5 min. The organic layer transferred to a 10ml clean glass tube and dried under a stream of nitrogen at 40 °C. After evaporation, each residue was reconstituted in 0.5ml of mobile phase and 20.0 µL was injected into the HPLC system for analysis.

A Younglin equipped with U.V/Vis. detector (model 370D) set at 223nm. and 5µm, C18 column (Reprosil 100) at 40°C were used in this experiment. The mobile phase consist acetonitril – ammonium acetate 0.05M (30 – 70) with a flow rate of 1.2ml/min.

Linearity was established by plotting the peak area ratios of florfenicol to chloramphenicol against the calibration voncentrations. Unknown sample concentrations were interpolated from the calibration curve.

Results

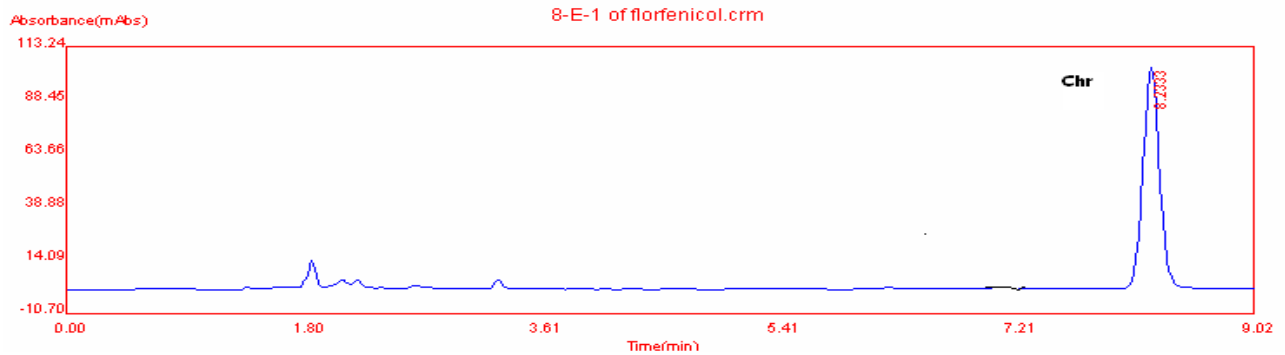
Chromatography and profile of plasma.

The chromatographs of a blank plasma containing I.S. (chloramphenicol) and standard plasma containing 2.5µg/ml florfenicol and a sample plasma obtained 2 hour after drug administration are shown in figure 1 (A, B, and C).

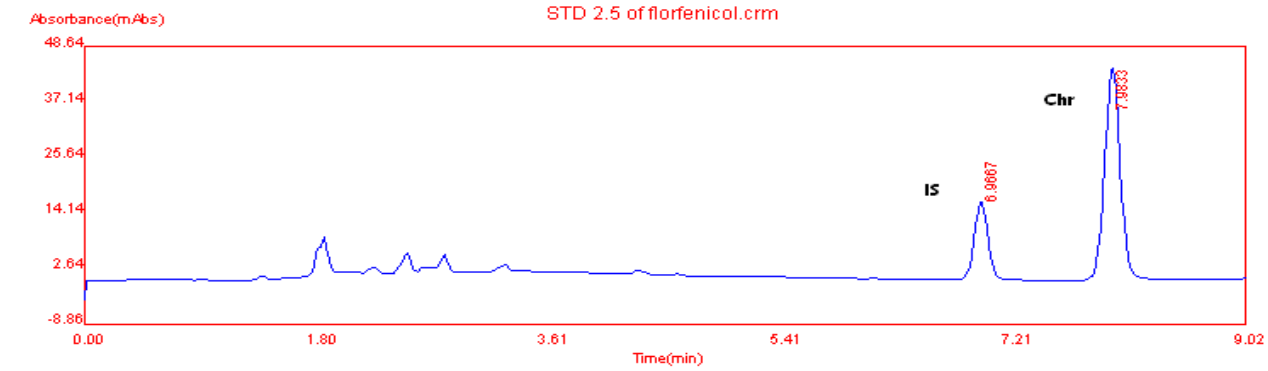
Figure 1;

HPLC Chromatographs of: **A-** blank plasma, **B-** Standard sample with 2.5µg/ml florfenicol and choramphenicl (as IS), **C-** Plasma sample 2 hours after drug administration

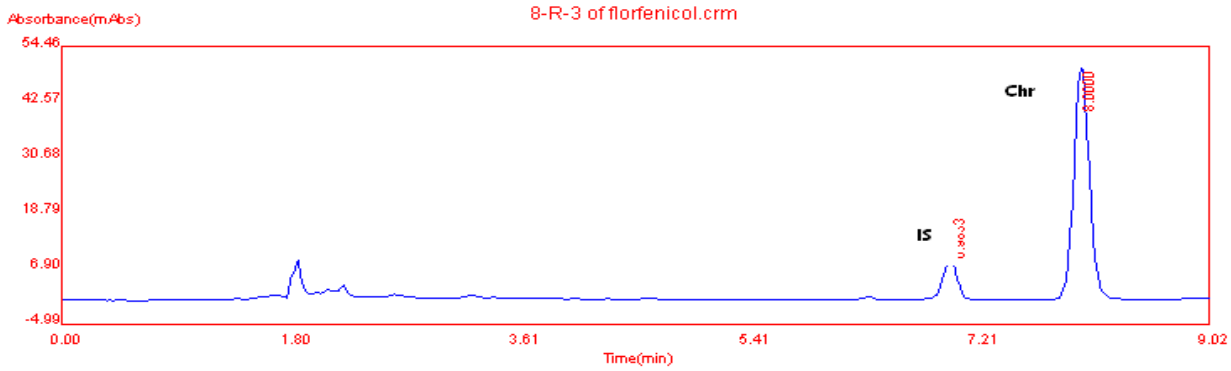
A



B



C



Method validation.

The standard curve of peak area ratios of florfenicol/choramphenicol vs. concentrations is shown in figure 2. Precision and accuracy of assay method is shown in table 1.

Table 1: Accuracy and precision in determination of Florfenicol concentrations in plasma sample by HPLC.

Conc. (µg/ml)	*N*	*Mean Measured Conc. ± S. D.*	*%C.V.*	%A
0.5	3	0.59 ± 0.54	9.16	18.74
1.0	10	1.03 ± 0.16	15.40	3.14
2.5	12	2.40 ± 0.23	9.44	-3.86
5.0	13	5.01 ± 0.4	7.9	0.15
10.0	14	9.81 ± 0.57	5.85	-1.91
20.0	5	20.33±0.22	1.08	1.63

Figure 2: Standard curve of florfenicol/chorfenicol peak area ratio vs concentration

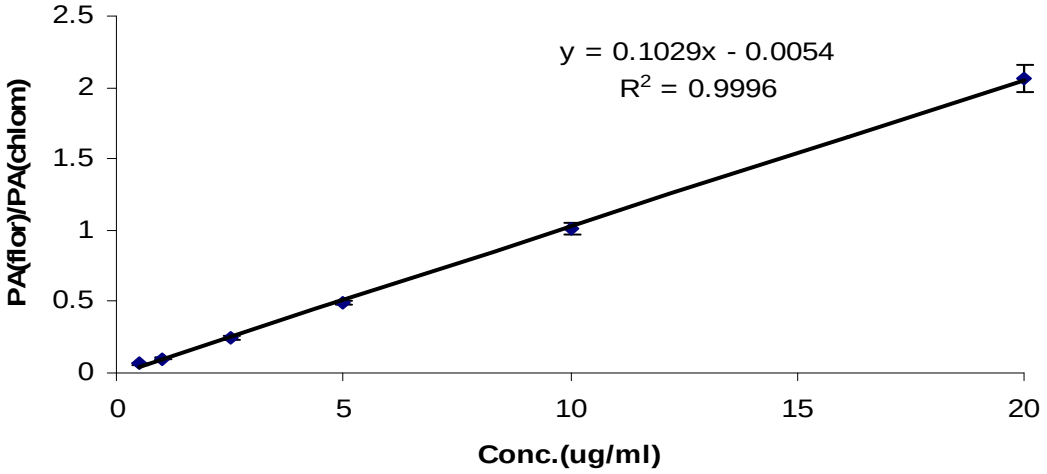
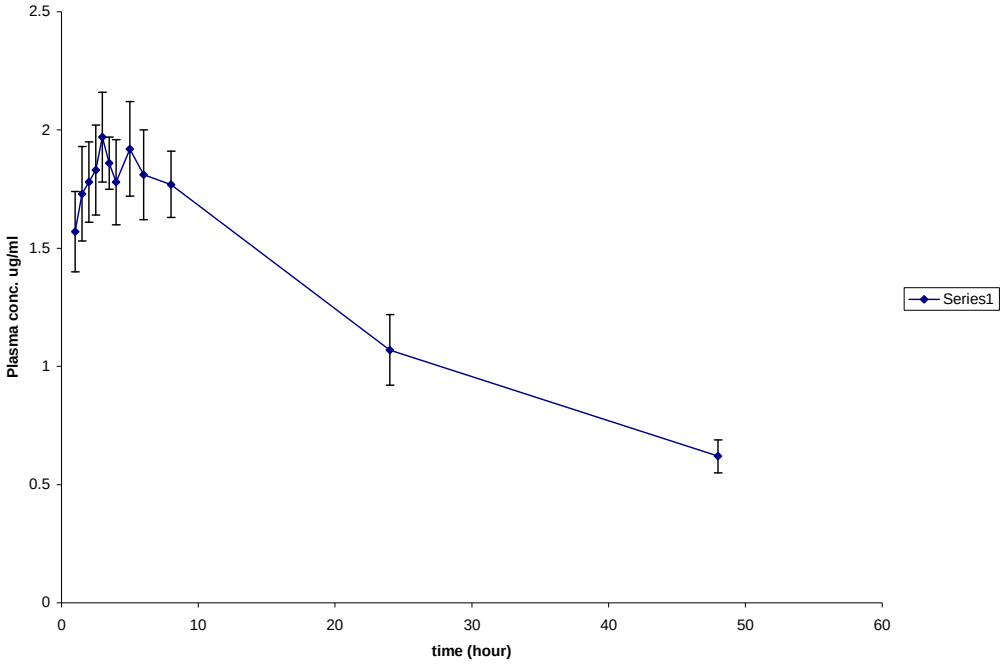


Figure 3: Florfenicol plasma concentration vs time after drug administration.



Pharmacokinetics of Florfenicol:
Plasma concentration of florfenicol after drug administration is shown in figure 3. Its pharmacokinetics parameter is shown in Table 2. The data reveals florfenicol reach its maximum concentration in about 4 hours (4.04±0.68).
A two compartment model best describe its pharmacokinetic with a $T_{1/2}$ of 2.871 h for distribution and 27.02 h for elimination phases (table 2).

Table 2: Pharmacokinetic parameters of Florfenicol in young male claves.

Parameter	*Mean*	*S.E.*
K_β Elimination (hr)	0.057	0.067
$T_{1/2}$ Elimination(hr)	27.02	3.81
K_α Distribution (hr)	0.377	0.06
T ½ Distribution(hr)	2.871	0.89
T_{max} (hr)	4.04	0.68
C_{max}(µ g/mL)	2.12	0.17
AUC_{0-48} (µg/mL.hr)	52.72	4.78
AUC_{0-inf} (µg/mL.hr)	87.22	9.64

Discussion:
Our results show this HPLC method is a précis and accurate method for determination of florfenicol in plasma and we used it to determine pharmacokinetic parameters in young male calves.
As can be seen from figure 2 the concentration of this drug is above 1µg /ml for more than 24 hours after its administration therefore it's active against various pathogens. The pharmacokinetics of florlorfenicol in young male calves same as other animal follows mulicompartemental pharmacokinetics.
We suggest to determine its pharmacokinetics in different sexes to clarify the effect of sex and milking effect on it.

Acknowledgement:
Authors would like to thanks Razak Pharmaceutical Co. Tehran – Iran for financial support of this project.

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