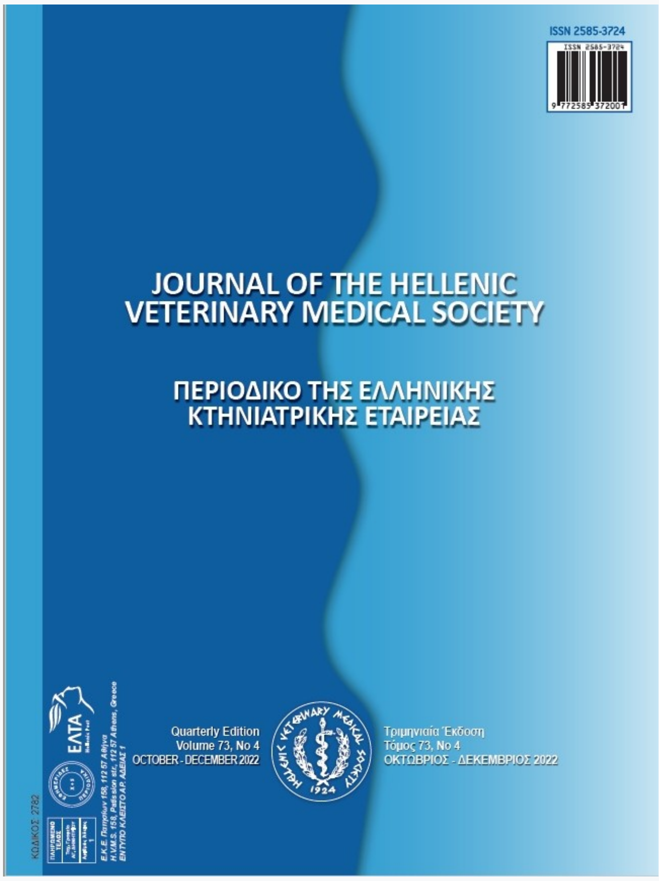
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 4 (2022)



Validation of a LC-UV method for determination of tildipirosin in goat milk

JS Galecio, V Hernandis, E Escudero, P Marín

doi: 10.12681/jhvms.28300

To cite this article:

Galecio, J., Hernandis, V., Escudero, E., & Marín, P. (2023). Validation of a LC-UV method for determination of tildipirosin in goat milk. *Journal of the Hellenic Veterinary Medical Society*, *73*(4), 4905–4912. https://doi.org/10.12681/jhvms.28300

J HELLENIC VET MED SOC 2022, 73 (4): 4905-4912
ΠΕΚΕ 2022, 73 (4): 4905-4912

Research article
Ερευνητικό άρθρο

VALIDATION OF A LC-UV METHOD FOR DETERMINATION OF TILDIPIROSIN IN GOAT MILK

J.S. Galecio[1,2], V. Hernandis[1,*], E. Escudero[1], P. Marín[1]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, University of Murcia, Campus de Espinardo, Murcia, Spain.

[2]Escuela de Medicina Veterinaria. Colegio de Ciencias de la Salud. Universidad San Francisco de Quito. Cumbayá, Ecuador

ABSTRACT: A simple, rapid, low-cost, and sensitive high-performance liquid chromatographic method was developed to determine tildipirosin in milk goat. Milk samples were precipitated with acetonitrile, and after evaporation, tildipirosin was determined by reverse-phase chromatography with an ultraviolet detector set at a wavelength of 289 nm. Tildipirosin was separated on aZorbax Eclipse XDB-C18 column, 150 x 3.0 mm, 5 μm with gradient chromatographic elution.The retention times for tildipirosin and tylosin tartrate were 4.4 min and 10.5 min, respectively. Calibration curves were ranged from 100 to 2500 μg/L. The lower limit of detection was 75 μg/L, and the lower limit of quantitation was 100 μg/L. The accuracy and precision were always <15% except for LOQ < 20%. Mean recovery was 95.6 %. This procedure can be applied to perform pharmacokinetic studiesin lactating animals and be useful to detect residues in dairy products.

Keywords: Goats, HPLC; Milk; Tildipirosin; Ultraviolet.

Corresponding Author:
Verónica Hernandis. Department of Pharmacology, Faculty of Veterinary Medicine, University of Murcia, Campus de Espinardo, 30.071-Murcia, Spain.
E-mail address: vhb@um.es.

Date of initial submission: 15-10-2021
Date of acceptance: 21-03-2022

INTRODUCTION

The frequent use of veterinary drugs in animal husbandry can result in occurrence of residues in drinking water and foodstuff, which can have serious adverse effects for consumers (Yu et al., 2017; Acaröz et al., 2021a). Antibiotic resistance has become a serious global problem and is steadily increasing worldwide in almost every bacterial species treated with antibiotics (Sengupta et al., 2013).This situation has been reached due to the abusive prescription of antibiotics, their inappropriate use by patients, and overuse of these substances in food industry(Capita& Alonso-Calleja, 2013; Van Boeckel et al., 2015).Despite this, veterinarians and doctors must apply them and provide effective treatments without further spreading of resistance. In this way, World Health Organization (WHO) establishes the concept of "One Health," where antimicrobial resistance is an ecological problem that is characterized by complex interactions involving diverse microbial populations affecting the health of humans, animals and the environment (WHO, 2015).

Tildipirosin (TD) is a semi-synthetic tylosin analog with a unique chemical structure characterized by two piperidine substituents on C20 and C23 and a basic mycaminose sugar moiety at C5 of the macrocyclic lactone ring. Owing to three nitrogen atoms accessible to protonation, TD is a tribasic molecule (Figure 1).This antimicrobial is exclusively used in veterinary practice, and it has been approved for parenteral treatment of respiratory disease in cattle and swine (Sengupta et al., 2013; EMA, 2016; Lei et al., 2018). Moreover, it shows favourable pharmacokinetic properties such as high apparent volume of distribution, high bioavailabilities after extravascular administration,and long half-lives (Galecio et al., 2020; Menge et al., 2012; Rose et al., 2013; Wang et al., 2018; Xiong et al., 2020). Before use in other species, the macrolide analysis in biological fluids is a pre-requisite step for dose optimization of TD therapy based on specific pharmacokinetic information in veterinary medicine, and its quantification in milk is necessary to detect and avoid the appearance of residues in dairy products. Because the milk provides many beneficial health effects; however, it can contain some hazardous substances such as antibiotic residues due to improper usage or misuse of antibiotics to livestock animals (Chen et al., 2019; Acaröz et al., 2021b).

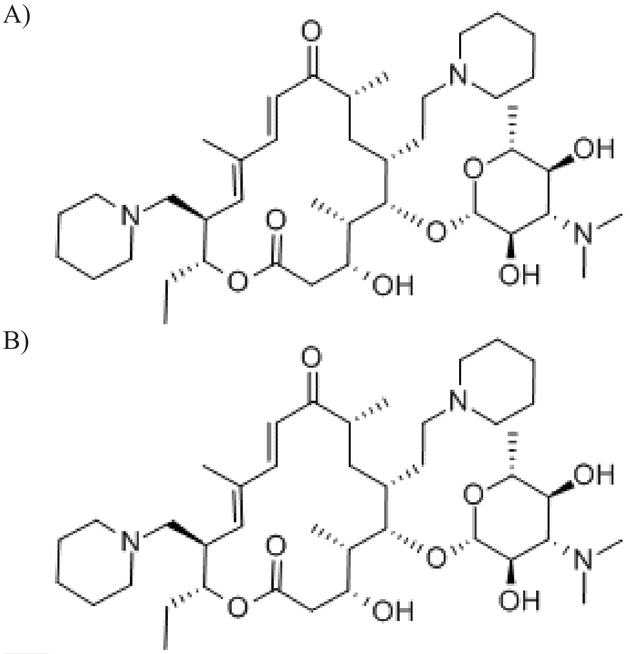


Figure 1. Chemical structure of tildipirosin (a) and internal standard tylosin (b)

Several detection methods including immunoassays, capillary electrophoresis, high-performance liquid chromatography, gas chromatography, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were developed for macrolide and aminoglycoside residue analyses with different sensitivities. However, LC-MS/MS is accepted as the most reliable confirmatory method based on its high sensitivity and accuracy (Dubois et al., 2001; Acaröz et al., 2020). One analytical method has been reported for measuring TD in biological bovine fluids (plasma, bronchial fluid, and lung tissue). This method involves sample preparation using solid-phase extraction (SPE) and separation and detection of TD using high-performance liquid chromatography (HPLC) coupled with mass spectrometry (LC ⁄ MS⁄ MS) (Mengue et al., 2013). Several authors have described the pharmacokinetics of this macrolide in different animal species using similar analytical methods (Abu-Basha et al., 2021; Wang et al., 2018;Zeng et al., 2018). Although these methods provide high sensitivity with a low limit of quantification values, they require sample preparation by SPE, which is relatively expensive due to the high number of samples to be analysed in pharmacokinetic studies. Besides, sample preparation and detection require sophisticated and expensive equipment LC/MS/MS, which is not commonly available in most laboratories. Up to date, only three specific HPLC methods with ultraviolet detection for TD determination in plasma have been reported (Abu-Basha et al., 2021; Elazab et al., 2020; Lei et al., 2018). In these methods, plasma samples were extracted with diethyl ether, obtaining limits of quantitationbetween10μg/L and 50μg/L and high recoveries. However, there is no published method for the determination and quantitation of TD in milk samples.

In near future, the pharmacokinetics of this drug will probably be widely studied in different dairy species. Therefore, the present study aimed to establish a simple, sensitive, and rapid HPLC method with UV detection to quantify TD in milk goat samples.

MATERIALS AND METHODS

Chemicals, solvents, and reagents

Tildipirosin and tylosin tartrate (internal standard) were obtained from CymitQuímica (Barcelona, Spain). All solvents were of HPLC analytical grade. Acetonitrile (ACN), formic acid (98-100%), ethanol 96%, and water were purchased by Merck Life Science (Madrid, Spain). Potassium di-Hydrogen Phosphate 99% and di-Potassium Hydrogen Phosphate 99% was supplied by PanReac AppliChem (Barcelona, Spain).

Instrumentation

The LC system consisted of an Agilent series 1220 Infinity (Agilent Technologies Spain, Madrid, Spain) with a dual gradient pump, a manual injector, a thermostatic column compartment, and a variable wavelength detector, all modules belonging to the 1220 series. The system mentioned above was connected to a Gilson 234 Autoinjector for HPLC systems (Gilson Incorporated, Middleton WI, USA).The chromatograms were recorded using Open Lab ChemStation software for the LC system (version A.01.05, Agilent, Spain).

Chromatographic conditions

Chromatographic separation was achieved using a Zorbax Eclipse XDB-C18 column (150 x 3.0 mm, 5 μm) from Agilent (Madrid, Spain) with an Eclipse XDB C18 (4.6 x 12.5 mm x 5μm) Agilent (Madrid, Spain) guard column. The mobile phase consisted of 0.3% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient elution profile was as follows: 0-13 min, 5-45% B; 13-14 min, 45-5 % B; 14-15 min, 5% B. The flow rate was 1.0 mL/min and the injection volume was 50 µL. The ultraviolet detection was made at a wavelength of 289 nm at 30º C. The retention time for TD and tylosin tartrate were 4.4 min and 10.5 min respectively. Finally, the run time was 15 min.

2.4 Samples collection

Seven clinically healthy Murciano-Granadina female lactating goats, without drug administration records in the last 60 days, were used to obtain the milk samples. These samples were obtained by manual milking until the mammary gland was empty and were taken around 09:00 in the morning (May 2021). The milk samples were stored at -40 ºC until assayed.

Standard solutions

Stock solutions of TD and internal standard (IS) were prepared at the concentration of 200 µg/mL. For TD, 20.24 mg of this macrolide were dissolved in 2 mL of ethanol (96%), and a solution of phosphate buffer 0.2 M (pH=7.4) was added into a 100 mL volumetric flask. The IS solution was prepared by the dilution of 28.63 mg of tylosin tartrate in 100 mL water for HPLC. The stock solutions were further diluted with water for HPLC to prepare working solutions at low concentrations. Working solutions of TD and IS in water were freshly prepared every week and refrigerated at 4°C.

Preparation of calibration curve and quality controls

Calibration curve (CC) and quality controls (QC) samples were prepared from a pool of goat blank milk spiked with an appropriate amount of TD working solution. After mixing, these samples were spiked with 10 µl of IS (200 µg/mL). Seven levels of concentration were used to compose the milk calibration curve range: 100, 250, 500, 750, 1250, 1875, and 2500 µg/L.Four levels of concentration were prepared for QCs; 100, 250, 1250 and, 2500 µg/L.

Sample preparation

Aliquots of 450 µL of milk were spiked with 10 µL of IS (200000 µg/L). After mixing, 900 µL acetonitrile was added. This solution was homogenized in a vortex for 1 min. After, the mixture were shaking in an ultrasonic bath for 5 min at 20°C, followed by centrifugation for 10 min at 1200 g. Supernatant was extracted, transferred to another polypropylene tube, and evaporated at room temperature (20°C) in a SpeedVac Vacuum Concentrators (Fisher Scientific, Madrid, Spain). The residue was reconstituted with 75 µL of the mobile phase, and 50 µL was injected into the HPLC system.

METHOD VALIDATION

Method validation was performed according to

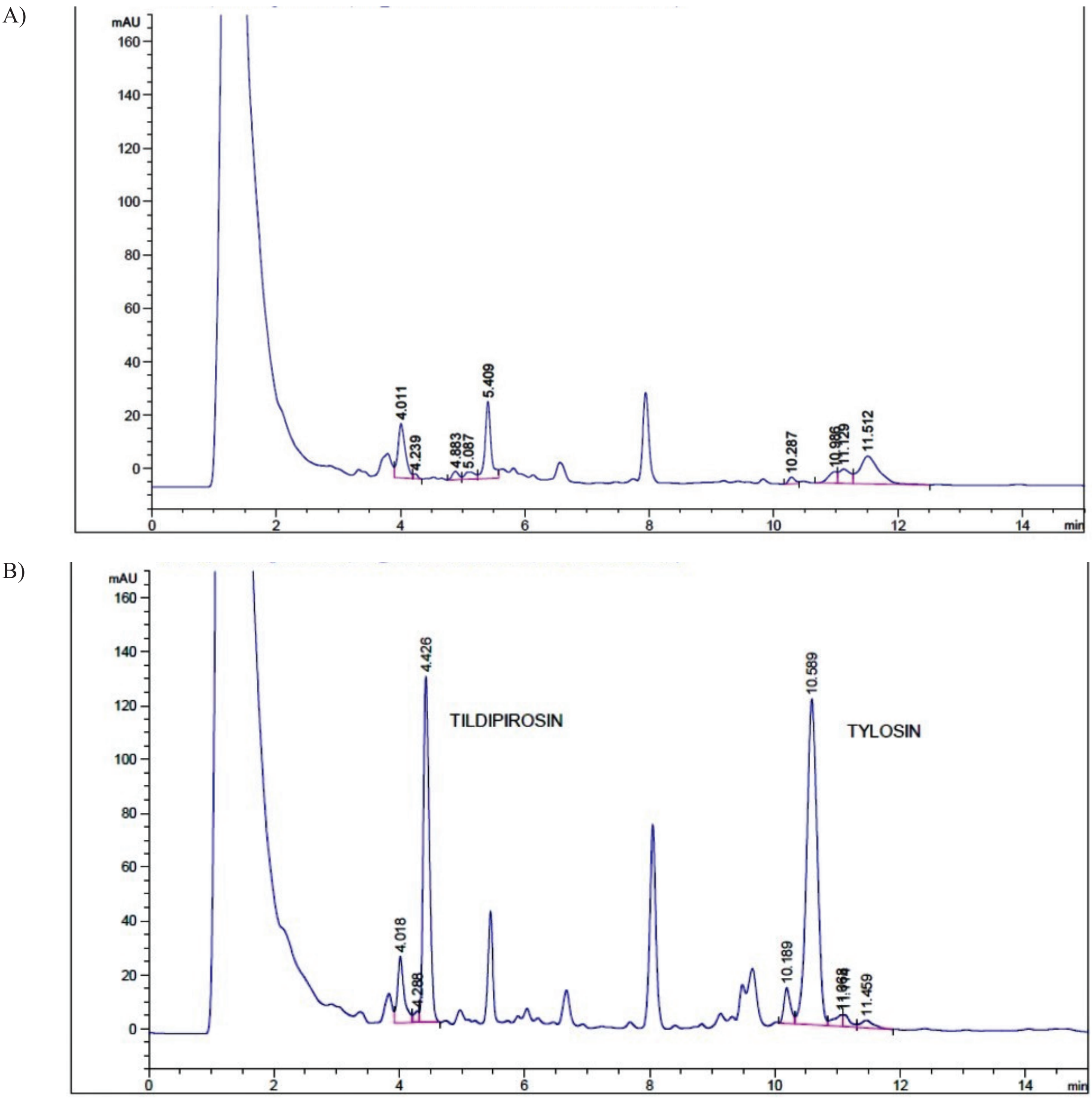


Figure 2. Chromatograms of tildipirosin and tylosin in goat milk by HPLC. (A) Blank milk; (B) Blank milk spiked with tildipirosin (2500 µg/L) and 10 µl of internal standard tylosin(200000 µg/L)

FDA guidelines for bioanalytical method validation (FDA, 2018). The following parameters were evaluated: linearity, lower limit of detection (LLOD), lower limit of quantitation (LLOQ), accuracy, precision, recovery, selectivity and, carry over.

Linearity, detection, and quantitation limits

The linearity of the proposed chromatographic method was examined by analysing seven concentrations of TD plus IS in goat milk samples. Calibration curves were obtained by plotting the ratio peak-area compound/IS versus known nominal concentrations of TD. Three replicates of each level were assayed. The lower limit of detection of TD was established as the concentration that provides a signal-to-noise ratio ≥ 3. The lower limit of quantitation was accepted as the lowest concentration on the calibration curve that can be determined with acceptable precision (coefficient of variation % ≤ 20).

Intraday and inter-day precision and accuracy

The precision and accuracy of the method were estimated by measuring of four (LOQ, low, medium, and high QCs)spiked with IS. Intra-day precision and accuracy were calculated on a single day using five replicates at each concentration level. Inter-day, precision and accuracy were evaluated using five replicates at each concentration level over three consecutive days. The accuracy was calculated with the following expression: ((measured concentration - nominal concentration)/ nominal concentration) x 100. The accuracy results should be ± 15% of nominal concentration, except ± 20 % at LOQ. For precision values, results should be ≤ 15% CV (variation coefficient) and ≤ 20% for LOQ.

Recovery

Recovery tests were analysed at three concentrations (low, medium, and high QCs). Five samples were assayed at each concentration level.Recovery (%) was calculated as: (mean peak area extracted milk samples / mean peak area extracted blank milk samples spiked with the analyte post-extraction) × 100.

Selectivity and carry over

The selectivity of this method was studied by analysing six samples of drug-free milk, free of interference at the retention times of TD and the IS. Potential injection carryover effects were excluded by analysing blank samples (n = 6) of milk directly after injection of a set of samples containing a high concentration of TD. The carryover effects should not exceed 20% of LOQ.

RESULTS

Linearity, detection and, quantitation limits

The peaks corresponding to TD and IS (Figure 2) were obtained at 4.4 min and 10.5 min respectively. Calibration curves were plotting by the area ratio of TD/ IS versus the nominal concentration of TD µg/L. The concentration range was linear from 100 µg/L to 2500 µg/L.A good linearity was observed ($r^2 \geq 0.995$). The results were $r^2= 0.9959$. The linear regression equations were found to be $y=3.0 \cdot 10^{-4} x+7.0 \cdot 10^{-4}$. The LOD and LOQ values were 75 and 100 µg/L. These values indicated that the proposed method is suitable and has adequate sensitivity for determining TD concentrations in milk by HPLC with ultraviolet detection.

Intraday and inter-day precision and accuracy

Precision and accuracy and results are shown in Table 1. Intra-day and inter-day precision were evaluated at four QCs concentration levels: 100, 250, 1250, and 2500 µg/L and five replicates measurements were recorded. The CV precision values in milk samples were < 9.0 % and <12.5 % for intra-day and inter-day precision, respectively. The same procedure was performed for intra-day and inter-day accuracy. The accuracy of the method for TD determination in milk samples was determined by replicate analysis of five sets of samples spiked with four different concentrations of TD: 100, 250, 1250 and, 2500 µg/L, and comparing the difference between the spiked value with the nominal value. The results are shown in Table I. The accuracy ranged from -14.7 % to 13.7 %. Relevant results were obtained, indicating that the method is reliable for the quantitative determination in milk goat samples.

Recovery

The recoveries of TD in milk were measured at low, mid and high QC levels (250, 1250 and, 2500 µg/L) by comparing extracted samples (n = 5) with blanks spiked with the analyte post-extraction. Mean recoveries at these three levels were from 92.5 ± 3.6 to 98.0 ± 12.0 %. The results are shown in Table 2. Excellent recoveries were obtained, indicating that this method was efficient.

Table 1. Intra-day and inter-day precision and accuracy of tildipirosin in goat milk samples

Intra-day	Nominal concentration (µg/mL)	Mean concentration± SD (µg/mL)	CV (%)	Accuracy (%)
	100	99.4 ±9.0	8.9	-0.5
	250	239.6 ±4.3	1.8	-4.1
	1250	1065.2 ± 12.2	2.1	-14.7
	2500	2190.4 ±105.9	4.8	-12.3
Inter-day				
	100	113.7 ± 20.3	8.2	13.7
	250	263.5 ± 19.9	12.4	5.4
	1250	1371.2 ± 164.1	5.9	5.0
	2500	2695.7 ± 307.9	1.7	7.8

Table 2. Mean ± SD recovery of tildipirosin in goat milk samples

Nominal Concentration (ug/L)	Recovery (%) (Mean ±SD)
250	92.5±3.6
1250	98.0±12.0
2500	96.2±10.7

Selectivity and carry over

Six blank milk samples were analysed and there were no endogenous interferences with the same retention times of TD and IS (Figure 2). Closer peaks at the retention time of tylosin is due to the impurities of this drug. Tylosin is a mixture of four closely related compounds: tylosin A, tylosin B, tylosin C, tylosin D. The mayor area of tylosin is tylosin A (80 %) (Arsic et al., 2018).These chromatograms were compared with spiked milk samples. Moreover, well-resolved peaks for TD and IS were observed. Adequate results were obtained, indicating the high selectivity of the method. Finally, the carryover effects have not been shown due to in six blank milk samples there were no peaks at the same TD retention time after running a set of milk samples with high concentrations of TD (n = 6).

DISCUSSION

This work aimed to achieve a satisfactory method for the determination of TD in goat milk samples with HPLC/UV detection. Four methods have been reported using HPLC-UV for TD detection in plasma (Abu-Basha et al., 2021; Elazab et al., 2020; Galecio et al., 2020; Lei et al., 2018; but a validated method for determining TD in goat milk samples by HPLC/UV have not been reported yet. Only one technique was published to determine this macrolide in bovine raw milk using HPLC high-resolution hybrid tandem mass spectrometry (LC-Q-Orbitrap) (Moretti et al., 2016). However, there are several methods to determine other macrolides, as azithromycin and tylosin, in milk in different species using LC/MS (Wang et al., 2020) and HPLC/UV detection (García-Mayor et al., 2006; Kurjogi et al., 2019;Nasr et al., 2014).Other authors have developed techniques by ultra-high-performance liquid chromatography-tandem mass spectrometry for more than sixty veterinary drugs in milk, although neither TD nor tylosin were included in these assays (Castilla-Fernández et al., 2019; Wang et al., 2018).It should be noted, mass spectrometry (MS) has become the technique of choice for the analysis of all macrolides in food, biological and environmental samples due to its sensitivity and specificity. HPLC/MS and HPLC/MS/MS are commonly used for milk samples due to the importance of detecting drug residues in these samples(Wang et al., 2017), but these sophisticated and expensive equipment LC/MS/MS is not commonly available in most laboratories. Moreover, LC/MS suffers from a high matrix effects that affect deeply in the accuracy, precision, and sensitivity (Zhou et al., 2017) that it is important to analyse.

Method development

For TD detection in pig, horse and goat plasma, different authors used diethyl ether as an extractant reagent(Abu-Basha et al., 2021; Elazab et al., 2020; Lei et al., 2018).However, our group research optimised a simple method to analyse TD in ewe plasma using acetonitrile as proteins precipitation with HPLC-UV detection (Galecio et al., 2020). Other authors also used this method in horse and rabbit plasma(Abu-Basha et al., 2021; Xiong et al., 2020)The use of acetonitrile for protein precipitation has been widely applied for the determination of different antibiotics and,different ratios have been proposed to achieve this propose (for example, a solution of plasma:acetonitrile (1:2 ratio)was sufficient to remove 99.4 % of proteins)(Blanchard, 1981).Besides, protein precipitation is one of the best fast methods to process biological samples (Ashri, Abdel-Rehim, 2011). Consequently, acetonitrile was used to reach an acceptable recovery in the present study. Other authors have analysed TDin plasma using HPLC/MS/MS and, it was extracted by solid-phase extraction (Abu-Basha et al., 2021). One disadvantage of this method is that this extraction process is time-consuming, and the use of cartridges and reagents significantly increases the price of each analysis.

Regarding the mobile phase conditions, the use of acidic mobile phases was the best option instead of preparing buffer solutions in HPLC/UV detection. The advantages of this mobile phase are a faster and simple preparation and a reduced tendency to precipitate and clog inside the chromatographic system. Moreover, gradient elution and isocratic elution were also investigated due to a longer time to IS elution than that for TD. Accordingly, gradient elution was chosen to obtain short running times.

Some usual C18 columns were checked in our laboratory: a Brisa LC^2,C18 column (150 mm x 4.6mm i.d x 5 µm) (Teknokroma, Barcelona, Spain),an ODS C18 column (250 mm x 4.6mm i.d x 5 µm) (Análisis-Vínicos, Tomelloso, Spain), a KromasilC18 column (250 mm x 4.6mm i.d x 5 µm) (Teknokroma, Barcelona, Spain) and a Zorbax Eclipse XDB-C18 column (150 x 3.0 mmi.dx5 µm) from Agilent (Madrid, Spain). Finally, the Zorbax Eclipse column was chosen as it showed the best resolution.

Validation

The obtained LLOQ value was 100 µg/L. This value was the same as that published in plasma of ewes (Galecio et al., 2020). Therefore, the proposed method is suitable and has adequate sensitivity for determining TD concentrations in goat milk samples. Other authors reported in horses(Abu-Basha et al., 2021)and pigs (Lei et al., 2018)lower LLOQ using HPLC/UV detection, but our technique showed other benefits,as faster and less costly than those mentioned above.

Excellent recoveries from 92.5 ± 3.6 to 98.0 ± 12.0%were obtained. Other authors have reported similar results for pig plasma (from 84 ± 1.02% to 102 ± 0.53%)(Lei et al., 2018). However, lower recoveries have been described in horse (from 79% to 82%)(Abu-Basha et al., 2021)and in ewe plasma samples(88.9 %) (Galecio et al., 2020) than in the present study. Moreover, the obtained intra-day and inter-day assays coefficients were < 20% for LLOQ and < 15% for low, medium, and high QCs. Consequently, our results indicate that this method was efficient and reproducible.

CONCLUSION

A novel, simple, rapid, sensitive, and reproducible method for the determination of TD in milk goat samples was developed by HPLC with ultraviolet detection, according to FDA guidelines. Furthermore, this validated method could be applied to clinical studies, routine analyses and, pharmacokinetic studies using a short running time.

ACKNOWLEDGEMENTS

Authors would like to thank the Fundación Carolina - España and Universidad San Francisco de Quito - Ecuador for the Ph.D. scholarship for Juan Sebastian Galecio.

CONFLICT OF INTEREST

None declared by the authors.

REFERENCES

Abu-BashaEA., Bani Ismail, Z, AbabnehMM, HamzehE, GehringR (2021) Pharmacokinetics and bioavailability of tildipirosin following intravenous and subcutaneous administration in horses.J Vet Pharmacol Ther00: 1-8.

Abu-BashaEA, Bani Ismail, Z, Abu Alhaijaa H, HamzehE, Idkaidek NM(2021). Pharmacokinetics and bioavailability of tildipirosin following intravenous and subcutaneous administration in sheep.J Vet Pharmacol Ther44: 79-85.

Acaröz U, Ince S, Arslan-Acaröz D, Küçükkurt I, Eryavuz A (2020). Determination of kanamycin residue in anatolian buffalo milk by LC-MS/MS. AnkUniv VetFakDerg26: 97-102.

Acaröz U, Ince S, Arslan-Acaröz D, Gürler Z, Kara R, Küçükkurt I, Eryavuz A (2021a)Evaluation of the enrofloxacin excretion in Anatolian buffalo milk. AnkUniv VetFak.Derg68: 121-127.

Acaröz U, Küçükkurt I, Ince S, Arslan-Acaröz D, Gürler Z, Eryavuz A (2021b)Assessment for the passage of tylosin into the milk of Anatolian buffaloes. J Hell Vet Medical Soc 72: 3127-3132.

Arsic B, Barber J, Čikoš A, Mladenovic M, Stankovic N, Novak P, (2018)16-membered macrolide antibiotics: a review; Int J Antimicrob Agents 51 (3): 283-298.

AshriNY, Abdel-RehimM(2011) Sample treatment based on extraction techniques in biological matrices.Bioanalysis 3: 2003-2018.

BlanchardJ(1981)Evaluation of the relative efficacy of various techniques fordeproteinizing plasma samples prior to high-performance liquid chromatographic analysis.J Chromatogr B Biomed Appl226: 455-460.

CapitaR, Alonso-CallejaC(2013)Antibiotic-Resistant Bacteria: A Challenge for the Food Industry. Crit Rev Food Sci Nutr53: 11-48.

Castilla-FernándezD, Moreno-GonzálezD, Beneito-CambraM, Molina-DíazA(2019)Critical assessment of two sample treatment methods for multiresidue determination of veterinary drugs in milk by UHPLC-MS/MS.Anal Bioanal Chem41: 1433-1442.

Chen J, YingGG, Deng W. J (2019)Antibiotic residues in food: extraction, analysis, and human health concerns. JAgricFood Chem 67: 7569-7586.

Dubois M, Fluchard D, Sior E, Delahaut P (2001) Identification and quantification of five macrolide antibiotics in several tissues, eggs and milk by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 753: 189-202.

ElazabST, BadawyME(2020)Pharmacokinetics and bioavailability of tildipirosin in goats using HPLC. Jpn J VetRes68: 5-12.

EMA (2016). Available online:
https://www.ema.europa.eu/en/medicines/veterinary/EPAR/zuprevo#authorisationdetails-section [accessed 16 March 2021].

Food and Drug Administration. U.S. Department of Health and Human Services, Bioanalytical Method Validation Guidance for Industry., U.S. Dep. Heal. Hum. Serv. Food Drug Adm. (2018) 1-41.
https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.[accessed 11April 2019].

García-MayorMA, GarcinuñoRM, Fernández-HernandoP, Durand-AlegríaJS(2006) Liquid chromatography-UV diode-array detection method for multi-residue determination of macrolide antibiotics in sheep's milk. J ChromatogrA1122: 76-83.

GalecioJS, EscuderoE, CerónJJ, CrescenzoG, MarínP(2020)Pharmacokinetics of tildipirosin in ewes after intravenous, intramuscular and subcutaneous administration. Animals10: 1-9.

KurjogiM, MohammadYHI, AlghamdiS, AbdelrahmanM, SataputeP, Jogaiah, S(2019) Detection and determination of stability of the antibiotic residues in cow's milk. PLos One14(10), e0223475.

LeiZ, LiuQ, YangB, AhmedS, CaoJ, HeQ(2018)The pharmacokinetic-pharmacodynamic modeling and cut-off values of tildipirosin against *Haemophilus parasuis.*Oncotarget9: 1673-1690.

MengeM, RoseM,BohlandC, ZschiescheE, KilpS, MetzW et al. (2012) Pharmacokinetics of tildipirosin in bovine plasma, lung tissue, and bronchial fluid (from live, nonanesthetized cattle). J Vet Pharmacol Ther35: 550-559.

MorettiS, CrucianiG, RomanelliS, RossiR, SalutiG, GalariniR(2016)Multiclass method for the determination of 62 antibiotics in milk.J. Mass Spectrom. 51: 792-804.

NasrJJ, ShalanS, BelalF(2014)Simultaneous determination of tylosin and josamycin residues in muscles, liver, eggs and milk by MLC with a monolithic column and time-programmed UV detection: application to baby food and formulae. Chem. Cent. J.8.

RoseM, MengeM, BohlandC, ZschiescheE, WilhelmC, KilpS. et al. (2013)Pharmacokinetics of tildipirosin in porcine plasma, lung tissue, and bronchial fluid and effects of test conditions on in vitro activity against reference strains and field isolates of *Actinobacilluspleuropneumoniae.*J Vet Pharmacol Ther36: 140-153.

SenguptaS, ChattopadhyayMK, GrossartHP(2013)The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol4: 47.

Van BoeckelTP, BrowerC, GilbertM, GrenfellBT, LevinSA, RobinsonTP, TeillantA, LaxminarayanR(2015)Global trends in Antimicrobial use in food animals. Proc NatlAcad Sci U.S.A112: 5649-5654.

WangH, RenL, YuX, HuJ, ChenY, HeG, JiangQ(2017)Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment.Food Control 80 :217-225.

WangJ, LeungD, ChowW, ChangJ, WongJW (2018)Target screening of 105 veterinary drug residues in milk using UHPLC/ESI Q-Orbitrap multiplexing data independent acquisition. AnalBioanal Chem41:5373-5389.

WangJ, LingY, ZhouW, LiD, DengY, YangX, ZhangF(2020)Targeted analysis of six emerging derivatives or metabolites together with 25 common macrolides in milk using Quick, Easy, Cheap, Effective, Rugged and Safe extraction and ultra-performance liquid chromatography quadrupole/electrostatic field orbitrap mass spectrometry.J Sep Sci 43: 719-3734.

WangJ, ZhaoT, SunX, LiuY, ZhuJ, ZhangSet al. (2018)Pharmacokinetics of tildipirosin in beagle dogs.J Vet Pharmacol Ther41: e49-e52.

WHO Global Action Plan on Antimicrobial Resistance, Microbe Mag. 10 (2015) 354-355.

XiongJ, XuY, HeS, ZhangY, WangZ, WangS, JiangH(2020)Pharmacokinetics and bioavailability of tildipirosin in rabbits following single-dose intravenous and intramuscular administration.J Vet Pharmacol Ther 43: 448-453.

Yu W, Knauer M, Kunas C, Acaröz U, Dietrich R, Märtlbauer E (2017) A gold nanoparticles growth-based immunoassay for detection of antibiotic residues. AnalMethods 9: 188-191.

ZengD, SunM, LinZ, LiM, GehringR, ZengZ(2018)Pharmacokinetics, and pharmacodynamics of tildipirosin against *Pasteurella multocida* in a murine lung infection model. Front Microbiol9: 1038.

Zhou W, Yang S, Wang PG(2017) Matrix effects and aplication of matrix effect factor.Bioanalysis9: 1839-1844.