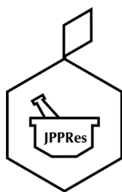

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Original Article

Development and validation of UHPLC method for the determination of fluazuron in bovine tissues

[Desarrollo y validación de método UHPLC para la determinación de fluazuron en tejidos bovinos]

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Abstract

Context: Fluazuron is an arthropod growth regulator drug for the control and treatment of parasitic infection in bovines by *Rhipicephalus (Boophilus) microplus*, one of the most important parasites for livestock due to the great economic losses.

Aims: To quantitative and confirmatory ultra-high-performance liquid chromatography with ultraviolet detection (UHPLC) method was developed to determine fluazuron in bovine tissues (muscle, fat, liver, and kidney) to assess drug residues.

Methods: Tissue samples were extracted with liquid phase extraction performed with acetonitrile. The chromatography (UHPLC) was performed using a C18 column (100 Å, 5 µm, 4.6 × 250 mm) maintained at 37°C. The mobile phase consisted of acetonitrile:water 65:35 v/v with a flow rate of 1.0 mL/min. The UV wavelength was set at 260 nm, and the injection volume was 100 µL for all worked tissues (injection site, muscle, fat, liver, and kidney).

Results: Validated analytical method exhibited a linear relationship over the range of 50–800 ng/g for muscle, including injection site, 125–2000 ng/g for kidney and liver, and 1750–28000 ng/g for fat, with a coefficient of determination greater than 0.99 in all cases. The lower limit of quantification (LLOQ) of fluazuron in muscle and injection site samples was 50 ng/g with a coefficient of variation (%CV) of 6.40%. The recovery percentage for fluazuron was greater than 75% in all the target tissues.

Conclusions: The extraction, separation, and quantification UHPLC method was valid to determine fluazuron in bovine tissues as well as for studies of drug residues in these animals.

Keywords: food-producing animals; quantification; residues; veterinary drugs.

Resumen

Contexto: Fluazuron es un fármaco regulador del crecimiento de artrópodos para el control y tratamiento de la infección parasitaria en bovinos por *Rhipicephalus* (*Boophilus*) *microplus*, uno de los parásitos más importantes para el ganado debido a las grandes pérdidas económicas.

Objetivos: Desarrollar un método de cromatografía líquida de ultra alta resolución cuantitativa y confirmatoria con detección ultravioleta (UHPLC) para la determinación de fluazuron en tejidos bovinos (músculo, grasa, hígado y riñón) en la evaluación de residuos de medicamentos.

Métodos: Las muestras de tejido se extrajeron con extracción en fase líquida realizada con acetonitrilo. La cromatografía (UHPLC) se realizó utilizando una columna C18 (100 Å, 5 µm, 4,6 × 250 mm) mantenida a 37°C. La fase móvil consistió en acetonitrilo:agua 65:35 v/v con un caudal de 1,0 mL/min. La longitud de onda UV se fijó en 260 nm y el volumen de inyección fue de 100 µL para todos los tejidos trabajados (lugar de inyección, músculo, grasa, hígado y riñón).

Resultados: El método analítico validado muestra una relación lineal en el rango de 50 a 800 ng/g para músculo, incluido el sitio de inyección, 125 a 2000 ng/g para riñón e hígado y 1750 a 28000 ng/g para grasa, con un coeficiente de determinación superior a 0,99 en todos los casos. El límite inferior de cuantificación (LLOQ) de fluazurón en muestras musculares y en el lugar de la inyección fue de 50 ng/g con un coeficiente de variación (%CV) de 6,40 %. En todos los tejidos diana el porcentaje de recuperación de fluazurón fue superior al 75%.

Conclusiones: El método UHPLC de extracción, separación y cuantificación fue válido para determinar fluazurón en tejidos bovinos, así como para estudios de residuos de fármacos en estos animales.

Palabras Clave: animales para la producción de alimentos; cuantificación; medicamentos veterinarios; residuos.

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INTRODUCTION

In livestock systems, parasitic disease constitutes one of the health problems that causes the greatest economic losses. The fight against parasitic diseases, and the diseases they transmit between animals mean in Uruguay a constant concern about direct losses and indirect effects that it causes mainly for the productive sector and animal health, attacking against the sustainability of the productive system in the commercial system (Fiel and Nari, 2013; Venzal et al., 2003). *Rhipicephalus* (*Boophilus*) *microplus*, known as the common cattle tick, is the tick with the greatest economic impact (Benavides and Romero, 2001), due to its wide distribution, vectoring capacity, bloodsucking habits, and the number of cattle it affects (Domínguez et al., 2016). Due to their great capacity for adaptation and propagation, ticks of the genus *Rhipicephalus* have been able to spread in various geographical areas of the world.

Bovine livestock constitutes one of the main protein sources of animal origin in the world. According to the US Department of Agriculture, in 2017, 23.5% of production was of bovine origin, surpassed by swine and poultry production (IDB, 2017). According to Bustillos (2014), 80% of the world's cattle are located in tropical and subtropical countries where ticks are active throughout the year. The problem for public health and productive systems depends on the context in which ticks and the diseases they transmit. Depending on the regions, the species, the transmitting agents, the number of the host population, the socioeconomic situation of the area, and technological advances in control measures, the seriousness of the problem takes a different magnitude. To a greater extent, the main economic losses generated by these ticks are due to actions for the control of cattle infestations, which predominantly use different chemical compounds, with varying degrees of toxicity and with a high economic cost (Bustillos, 2014).

Up to the present, chemical treatments have been an affordable and effective control tool, but the average time from introducing a new acaricide until it develops resistance or is withdrawn from the market is only about eight years (Playford et al., 2005). For its parasitic control, one of the alternatives is to interrupt the development of the biological cycle of the tick through the application of antiparasitic drugs, such as fluazuron (Junquera et al., 2019; Velázquez et al., 2016). At present, there are different commercially available pharmaceutical alternatives (topic or injectable) for the control of ticks in cattle.

The use of antiparasitic drugs to achieve tick control or eradication needs to be monitored regarding drug residues on edible tissues (Canton et al., 2021). This article proposes developing and validating the UHPLC analytical method for quantifying fluazuron in bovine tissues (injection site, muscle, fat, liver, and kidney) to assess drug residues.

MATERIAL AND METHODS

Ethical considerations

The experimental study was approved by the Comisión Honoraria de Experimentación Animal, Universidad de la República, Uruguay (CEUAFVET-943 111900-000887-19).

Analytical procedures

Chemicals and reagents

HPLC grade solvents acetonitrile was used, as well as Milli-Q® ultrapure water that was previously filtered using a hydrophilic 0.45 µm membrane filter. Solid standards of fluazuron and prazepam were used to prepare standard and working solutions. Prazepam was selected as the internal standard, and fluazuron was the analyte of interest.

Drug extraction

Extraction methods reported in the literature for fluazuron were previously analyzed (Ferreira et al., 2019). Prior to the extraction process, muscle, fat and kidney samples were fortified with prazepam 820 ng/mL. Fortification with 50 µL was performed for muscle and fat samples, and 100 µL was used for kidney and liver samples. Prazepam was the internal standard for quantification by UHPLC of fluazuron in tissue samples. The processing of the injection site samples was identical to that of the muscle samples.

A liquid phase extraction was performed using acetonitrile: 2 mL of solvent was added to 1 g tissues samples processed by grating, and the mixture was placed in a vortex shaker for 1 minute (DMT-2500 Multi-Tube Vortex Mixer, Miulab, Hangzhou Miu Instruments Co., Ltd., China). After centrifugation for 3 min at 3000 rpm (refrigerated centrifuge 2-16KL, Sigma, Germany)., the supernatant was transferred to a 2.5 mL plastic syringe and filtered with 0.45 µm Minisart® regenerated cellulose hydrophilic filter. The filtrate was transferred to a 5 mL vial, evaporated to dryness at 55°C, and redissolved in 300 µL of mobile phase for direct injection into the UHPLC column.

Chromatographic conditions

Quantification methods for fluazuron by UHPLC available in the scientific literature were studied to develop and optimize the mobile phase composition (Armishaw et al., 1996; Zhang et al., 2013) and the running conditions (Ferreira et al., 2019). HPLC-UV system consisted of a Dionex Ultimate 3000 system separation module coupled to a Dionex Ultimate 3000 UV-Vis detector and a UHPLC Ultimate 3000 quaternary gradient pump, controlled by the Chromeleon software from Dionex. The chromatography was performed using a C18 column (100 Å, 5 µm, 4.6 × 250 mm) maintained at 37°C. The mobile phase consisted of acetonitrile:water 65:35 v/v with a flow rate of 1.0 mL/min for muscle, liver, and kidney samples and 0.5 mL/min for fat samples. The flow for the fat samples was adjusted to allow a better chromatographic separation of the internal standard and impurities of the tissue. The UV wavelength was set at 260 nm, and the injection volume was 100 µL for all worked tissues (injection site, muscle, fat, liver, and kidney).

Method validation

The proposed method was validated in accordance with elements of EMEA 2011 (EMEA, 2011) using blank tissue samples and spiked samples at different concentrations. Also, according to the International Conference on Harmonization (ICH, 1995), the internal standard should be structurally similar to the main compound of analysis.

Linearity

Linearity was evaluated on six levels of known concentration samples by triplicates, on three different days, prepared in the concentration range of analysis for each tissue: 125-2000 ng/g for kidney and liver, 1750-14000 ng/g for fat, 50-800 ng/g for muscle and injection site. Concentration ranges for every tissue were selected to include the residue levels determined by EMEA (EMEA, 2005; 2018). Calibration curves were generated by plotting the relative peak areas as a function of the fluazuron sample concentration (ng/g) on three different days and three replicates for each concentration. Requirements for a valid calibration model were a regression coefficient (R^2) higher than 0.990 and a coefficient of variation (CV) to be within ± 20% at the lower limit of quantification (LLOQ) and ± 5% for the rest of the concentrations tested.

Precision and accuracy

Samples were prepared in one concentration level per tissue with six replicates each. The precision was characterized by the percentage coefficient of variation [CV (%)], and the accuracy was measured as a percentage of recovery [R (%)] calculated between the concentration calculated by interpolation from the calibration curve and the theoretical concentration for each of the samples. The acceptable precision limit is considered to be 5% for biological samples expressed as a coefficient of variation in percentage. Regarding the accuracy of the method, a recovery percentage in the 90-110% range is considered acceptable (WHO, 2002).

Selectivity

Selectivity was proved by comparing chromatograms of six blank tissue samples with tissue samples spiked with fluazuron. Interference was tested, and selectivity was ensured at the lower limit of quantification. The accepted limit for interference was a percentage difference in the ratio of relative areas less than 20% of the lower limit of quantification for the analyte.

Carry over

During the validation process, carry over was determined by injecting blank samples after being injected with an upper limit of analyte (ULOQ) quantification on three replicates. Carry over on blanks should not be more than 20% of LLOQ (lower limit of quantification per tissue).

Method application in clinical samples

Eight male Holstein calves were euthanized captive-bolt shot at 135 days after administration of fluazuron (fluazuron 12.5%, 12.5 mg/kg + ivermectin 1%, 0.2 mg/kg, by subcutaneous route). Animal tissue samples (injection site, muscle, fat, kidney, and liver) were collected on site and stored at -20ºC until analysis was performed (VICH, 2002).

Statistical analysis

Statistical analysis of data obtained during a method validation was performed to demonstrate the validity of the analytical method. The linearity was estimated by linear regression analysis by the least square regression method (95% confidence intervals). P-values less than 0.05 were considered significant. All analyzes were performed in R software (version 4.1.2) (R Core Team, 2022; RStudio Team, 2022).

RESULTS AND DISCUSSION

Selectivity

Selectivity was evaluated by processing three different blank tissue samples. The absence of interfering peaks at the retention time of fluazuron in each target

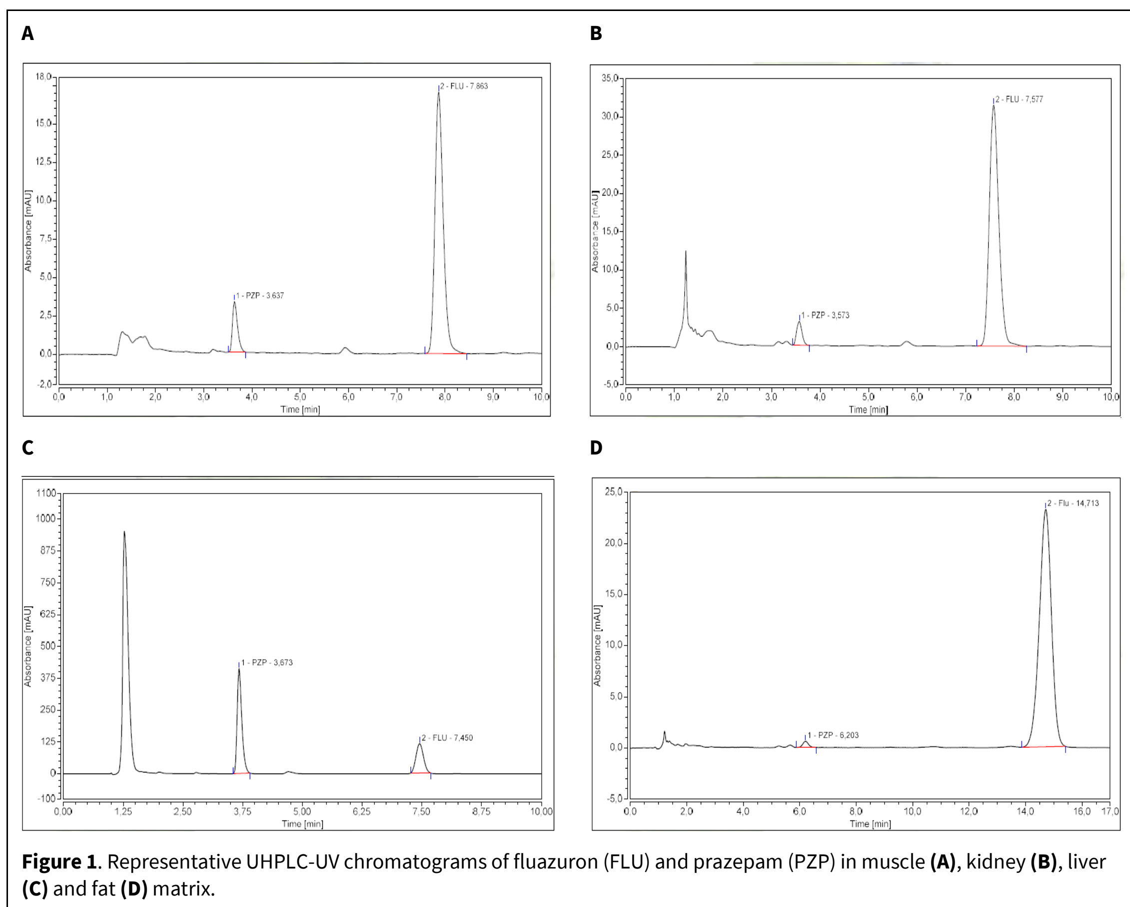

Figure 1. Representative UHPLC-UV chromatograms of fluazuron (FLU) and prazepam (PZP) in muscle **(A)**, kidney **(B)**, liver **(C)** and fat **(D)** matrix.

tissue was considered as evidence for the selectivity of the experimental method. The chromatograms of fluazuron and its respective internal standard in each tissue are shown in Fig. 1A-D.

Calibration curves (linearity and ranges)

The calibration curves of fluazuron in bovine tissues were obtained based on the peak area ratio of fluazuron to internal standard *vs.* the fluazuron concentration, exhibiting a linear relationship over the range of 50–800 ng/g for muscle and injection site (y = 37.3 [95% CI 36.5, 38.0]x + 4.26 [95% CI -4.5, 13.0]), 125–2000 ng/g for kidney (y = 28.7 [95% CI 28.1, 29,3]x – 21.6 [95% CI -41.9, -1.31]) and liver (y = 43.1 [95% CI 43.0, 43.2]x – 11.2 [95% CI -13.3, -9.05]) and 1750–28000 ng/g for fat (y = 43.7 [95% CI 42.8, 44,5]x - 294 [95% CI -424, -165]). The lower limit of quantification (LLOQ) of fluazuron in muscle and injection site samples was 50 ng/g with a coefficient of variation (%CV) of 6.40%.

The fluazuron standard calibration curve (95% CI) and equations of linear regression analysis were obtained for all tissue targets, as shown in Fig. 2 and Table 1, respectively.

Recovery

Recovery was calculated considering mobile phase standard solutions prepared at the theoretical concentration of fluazuron in each tissue, assuming 100% recovery. The recovery percentage for fluazuron was greater than 75% in all target tissues, with the lowest percentage in muscle (75.5%) and the highest in fat (98.6%). The extraction method is considered to be valid for all tissues and has a high recovery percentage.

Carry over

The carry over test results did not exceed the requirements of the analytical method (not greater than 20% of the LLOQ) for all bovine tissues under study.

Precision and accuracy

Table 2 presents the values and limits of precision and accuracy established for our study. The chromatographic area results obtained for accuracy showed a CV of less than 5% for the concentration range presented in each target tissue. Regarding the accuracy of the method, the CVs achieved for the percentage recovery are considered adequate (range 90-110%).

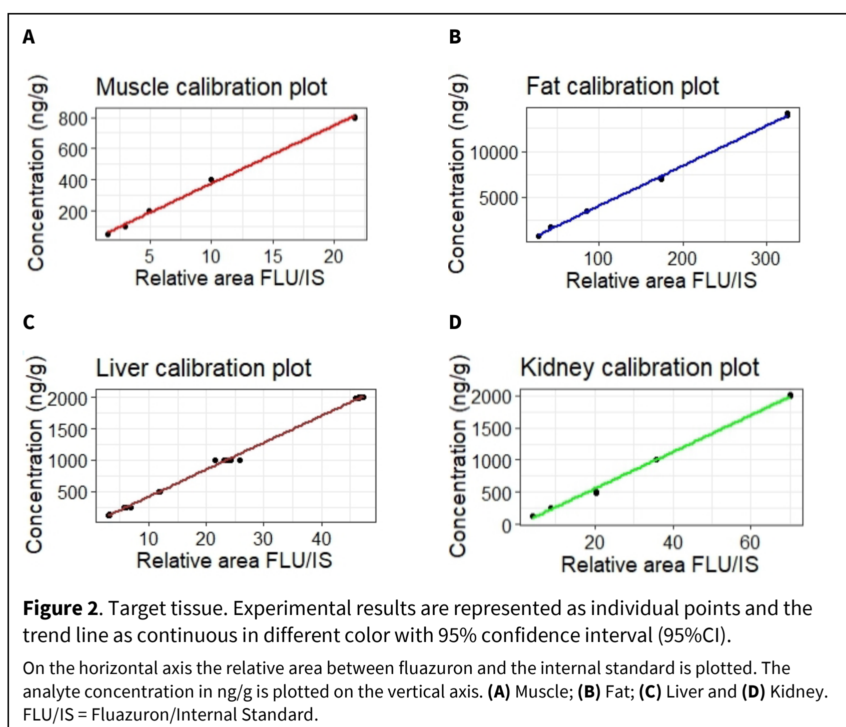

Figure 2. Target tissue. Experimental results are represented as individual points and the trend line as continuous in different color with 95% confidence interval (95%CI).

On the horizontal axis the relative area between fluazuron and the internal standard is plotted. The analyte concentration in ng/g is plotted on the vertical axis. **(A)** Muscle; **(B)** Fat; **(C)** Liver and **(D)** Kidney. FLU/IS = Fluazuron/Internal Standard.

Table 1. Linear regression analysis of fluazuron standard calibration curve in different target tissue.

Parameters	Muscle	Fat	Liver	Kidney
Intercept (ng/g)	4.26	-294.78	-11.17	-21.59
(95% CI)	(-4.49, 13.01)	(-424.36, -165.21)	(-13.29, -9.04)	(-41.87, -1.31)
Area ratio	37.25	43.68	43.08	28.69
(95% CI)	(36.45, 38.04)	(42.83, 44.52)	(42.99, 43.16)	(28.11, 29.27)
Nº observations	30	28	30	29
R^2	0.997	0.998	1.000	0.997
R^2 adjusted	0.997	0.998	1.000	0.997
AIC	253.8	384.7	167.9	292.2
BIC	258.0	388.7	172.1	296.3
Log Likelihood	-123.9	-189.4	-80.9	-143.1
F	9162.9	11295.9	1007211.2	10226.2
RMSE	15.0	209.3	3.6	33.6

Application in clinical samples

In all animals and target tissues, no concentrations higher than maximum residue levels (MRLs) were detected for each of the tissues studied (Table 3). The results obtained allow consideration that the correct application and according to the specifications of use of the product is safe in terms of risk and is legally tolerated in animal tissues. No differences between target tissues in the presence of fluazuron concentrations were visualized, confirming the consistency in the withdrawal time established by governmental authorities for fluazuron (retention periods = 135 days). The method is effective for the proposed objective and represents a faster and lower-cost work methodology than other determinations previously published in the scientific literature (Ferreira et al., 2019; Teixeira et al., 2021). The detection and quantification limits of the developed UHPLC-UV method are markedly higher than those determined by mass spectrometry that have been published (Suárez et al., 2021; Yoo et al., 2020). The method can be refined to reach lower limits of quantification and be used to determine plasmatic concentrations. However, it was not necessary in the present work due to the determination of objectives.

Table 2. Shows the results of precision and accuracy of fluazuron in each bovine target tissue.

Tissue	Fluazuron theoretical concentration (ng/g)	Precision (% CV)	Accuracy (% R)
Muscle	200	1.94	102.6
Fat	7000	2.20	107.9
Liver	500	1.32	99.4
Kidney	500	1.50	92.2

Table 3. Determination maximum residue levels (MRLs) of fluazuron in target tissue of animals dosed with fluazuron (12.5 mg/kg, subcutaneous) after 135 days from treatment.

Target tissues	Fluazuron concentration (ng/g)	MRLs* (µg/kg)	LOQ
Muscle	< LOQ	200	50
Fat	< LOQ	7000	1800
Liver	< LOQ	500	125
Kidney	< LOQ	500	125
Site injection	< LOQ	200	50

*EMEA/CVMP/77290/05-FINAL (EMEA, 2005).

CONCLUSION

The UHPLC quantification method with UV detection was valid for determining fluazuron in bovine tissues (muscle, liver, kidney, and fat). It demonstrated suitable chromatographic parameters, standing out among selectivity, linearity, sensitivity, precision, accuracy, and extraction recovery percentage for the analyte of interest. The developed method can be used in pharmacokinetic determinations of fluazuron in bovine tissues as well as for studies of drug residues in these animals.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exists with the data contained in this paper. The selection of the approved formulation (Animal Drugs Product from Ministerio de Agricultura y Pesca [MGAP] in Uruguay) did not respond to any particular interest of authors.

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REFERENCES

Armishaw P, Ward J, Millar RG (1996) Development of a reference material for residues of chlorfluazuron and fluazuron in beef fat ACSL CRM 3. Fresenius J Anal Chem 356(1): 10–12. https://doi.org/10.1007/s0021663560010

Benavides E, Romero N (2001) Consideraciones para el control integral de parásitos externos del ganado. Rev Carta Fedegán 70: 64–86.

Bustillos RC (2014) Ecología parasitaria de la garrapata (acari: ixodidae) en bovinos en dos áreas geográficas del Ecuador. Quito: UCE, p. 63.

Canton L, Lanusse C, Moreno L (2021) Rational pharmacotherapy in infectious diseases: Issues related to drug residues in edible animal tissues. Animals 11(10): 2878. http://dx.doi.org/10.3390/ani11102878

Domínguez D, Torres F, Rosario-Cruz R (2016) Evaluación económica del control de garrapatas *Rhipicephalus microplus* en México. Rev Iberoam Cienc Biol Agropec 5(9): 43–52.

EMEA - European Medicines Agency (2011) Committee for medicinal products for human use (CHMP). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf [Consulted October 29, 2022].

EMEA - European Medicines Agency (2018) Committee for medicinal products for veterinary use. European public MRL assessment report (EPMAR) Fluazuron (All ruminants, except bovine and ovine, and fin fish). EMA/CVMP/456716/2017 https://www.ema.europa.eu/en/documents/mrl-report/fluazuron-all-ruminants-except-bovine-ovine-fin-fish-european-public-maximum-residue-limit_en.pdf [Consulted November 10, 2022].

EMEA - European Medicines Agency (2005) Committee for medicinal products for veterinary use. Fluazuron summary report. EMEA/CVMP/77290/05-FINAL. https://www.ema.europa.eu/en/documents/mrl-report/fluazuron-summary-report-committee-veterinary-medicinal-products_en.pdf [Consulted November 10, 2022].

Ferreira T, Lima I, Magalhaes V, Avelar B, Oliveira G, Barbour Scott F, Cid Y (2019) Bioanalytical method to measure fluazuron in bovine plasma and its application in pharmacokinetic studies.

Rev Virtual Quím 11: 1067–1079. https://doi.org/10.21577/1984-6835.20190072

Fiel C, Nari A (2013) Enfermedades parasitarias de importancia clínica y productiva en rumiantes: Fundamentos epidemiológicos para su diagnóstico y control. Montevideo, Hemisferio Sur, 1, p. 751.

ICH - International Conference on Harmonization (1995) Guidance for Industry, Q2B: Validation of Analytical Procedures: Methodology. USA: International Conference on Harmonization.

IDB - Interamerican Development Bank (2017) La producción mundial de carnes: La transición nutricional y el protagonismo de los emergentes. https://conexionintal.iadb.org/2017/06/02/la-produccion-mundial-carnes-la-transicion-nutricional-protagonismo-los-emergentes [Consulted November 10, 2022].

Junquera P, Hosking B, Gameiro M, Macdonald A (2019) Benzoylphenyl ureas as veterinary antiparasitics. An overview and outlook with emphasis on efficacy, usage and resistance. Parasite 26: 26. https://doi.org/10.1051/parasite/2019026

Playford M, Rabiee AR, Lean IJ, Ritchie M (2005) Review of research needs for cattle tick control, Phases I and II. Meat & Livestock Australia Ltd., Sydney. https://www.mla.com.au/research-and-development/reports/2005/review-of-research-needs-for-cattle-tick-control---phases-i-and-ii/ [Consulted November 10, 2022].

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ [Consulted July 14, 2022].

RStudio Team (2022) RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com [Consulted July 14, 2022]

Suárez G, Robaina D, Muela A (2021) Is the suckling period and application pattern relevant for fluazuron against tick infestation in cows and their suckling calves? BMC Vet Res 17: 375. https://doi.org/10.1186/s12917-021-03090-7

Teixeira RA, Dinali LAF, Silva CF, de Oliveira HL, da Silva ATM, Nascimento CS, Borges KB (2021) Microextraction by packed molecularly imprinted polymer followed by ultra-high performance liquid chromatography for determination of fipronil and fluazuron residues in drinking water and veterinary clinic wastewater. Microchem J 168: 106405. https://doi.org/10.1016/j.microc.2021.106405

Venzal JM, Castro O, Cabrera PA, Souza C G de, Guglielmone AA (2003) Las garrapatas de Uruguay: Especies, hospedadores, distribución e importancia sanitaria. Veterinaria (Montevideo) 38(150-151): 17–28.

Velázquez Terra R, Otegui Riccetto MA (2016) Caracterizar el modelo de infección artificial de *Rhipicephalus* (*Boophilus*) *microplus* en bovinos y determinación de la eficacia ectoparaciticida de una formulación de ivermectina 3, 15% larga acción. Tesis de grado, Universidad de la República, Facultad de Veterinaria. https://www.colibri.udelar.edu.uy/jspui/handle/20.500.12008/10412

VICH (2002) Food and Drug Administration, HHS. International Cooperation on Harmonisation of Technical Requirements for Approval of Veterinary Medicinal Products (VICH); final guidance on "Safety studies for veterinary drug residues in human food: reproduction toxicity testing" (VICH GL22); availability. Notice Fed Regist 67(3): 603–605.

WHO (2002) 36th Technical Report - Annex 3 of WHO Technical Reports Series, No. 902.

Yoo KH, Park DH, Abd El-Aty AM, Kim SK, Jung HN, Jeong DH, Cho HJ, Hacimüftüoğlu A, Shim JH, Jeong JH (2020) Development of an analytical method for multi-residue quantification of 18 anthelmintics in various animal-based food products using liquid chromatography-tandem mass spectrometry. J Pharm Anal 11(1): 68–76. https://doi.org/10.1016/j.jpha.2020.03.008

Zhang Y, Lu X, Hu C, Cui W, Liu X (2013) Development of a high performance liquid chromatography method for quantification of fluazuron in cattle tissues. J Liquid Chromatogr Related Technol 36(18): 2559–2567. https://doi.org/10.1080/10826076

AUTHOR CONTRIBUTION:

Contribution	Valiante C	Robaina D	Alvariza S	Suárez V
Concepts or ideas			x	x
Design		x	x	x
Definition of intellectual content			x	x
Literature search	x	x	x	x
Experimental studies	x		x	
Data acquisition	x		x	
Data analysis	x		x	x
Statistical analysis	x			x
Manuscript preparation	x	x	x	x
Manuscript editing	x			x
Manuscript review	x	x	x	x

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