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Pharmacokinetics of Cannabidiol and its Metabolites for the Definition of Ineffective Plasma and Urinary Concentrations in Biological Liquids of Sport Horses

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ABSTRACT

Cannabidiol (CBD) is increasingly used in veterinary medicine, although its pharmacokinetics in horses remain variable due to the heterogeneity of the available oral CBD formulations. As a substance controlled by regulatory bodies, CBD demands clear guidelines to distinguish pharmacologically active concentrations from irrelevant residues. This study evaluated the pharmacokinetics of a CBD oral formulation and its major metabolites (7-hydroxy-cannabidiol - 7OH-CBD and 7-carboxy-cannabidiol - 7COOH-CBD) in horses, defining also its pharmacologically irrelevant plasma (IPC) and urinary (IUC) concentrations. Six standardbred mares received a single 200mg CBD oral dose. Plasma and urine were collected before administration and up to 120 hours and analyzed using LC/HRMS. Pharmacokinetic parameters were determined applying non-compartmental analysis and IPC-IUC were calculated with Toutain and Lassourd's pharmacokinetic/pharmacodynamic approach. CBD exhibited a short half-life (2.52±1.54h) and low C_{max} (1.98±1.29ng/mL), reflecting extensive first-pass metabolism and high interindividual variability. Conversely, 7COOH-CBD displayed a prolonged half-life (32.47±9.51h) and higher systemic exposure, whereas 7OH-CBD was not quantified in plasma. The calculated IPC and IUC for CBD were 0.37pg/mL and 31.36pg/mL, respectively, with a withdrawal time (WT) of 1.2 days. For 7COOH-CBD, the IPC was 158.29pg/mL, IUC 55.40pg/mL, and WT 10.5 days. No THC was detected in any samples. These findings provide thresholds for CBD and 7COOH-CBD following administration of this oral formulation and support rational WT for equine athletes. The prolonged detectability of 7COOH-CBD highlights the need for regulations distinguishing active from irrelevant residual concentrations to ensure fair competition.

Keywords: Cannabidiol, Doping control, Irrelevant plasma concentration, Irrelevant urinary concentration, Pharmacokinetics, Sport horses.

INTRODUCTION

Cannabidiol (CBD), a non-psychoactive compound from *Cannabis sativa*, has seen rising use in veterinary medicine, particularly for companion and performance animals (Kogan et al. 2019; Ukai et al. 2023; Temmerman 2025). In horses, CBD has emerged as a potential therapeutic option for conditions such as chronic pain, inflammation, and stress-related behavioural issues, despite the limited scientific data on its efficacy (Draeger et al. 2021; Turner et al. 2021a). The CBD effects are mediated through its interaction with the endocannabinoid system (ECS) and other receptors (TRPV1, GPR55, serotonin receptors), which regulate pain, inflammation, and homeostasis (Kupczyk et al. 2022). In horses, ECS receptors have been reported to be distributed in the central nervous system, osteoarticular tissues, and the hoof, thus representing potential therapeutic targets in response to

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CBD administration (Miagkoff et al. 2023; Zamith Cunha et al. 2023a; 2023b; Bombardi et al. 2025; Zamith Cunha et al. 2025).

However, unlike in humans and dogs where pharmacokinetic profiles are better established, equine-specific data remain sparse due to variations in formulation, route of administration, and analytical approaches (Luedke & Wilhelm 2021; Williams et al. 2022; Yocom et al. 2022; Eichler et al. 2023; Trevisiol et al. 2025; Wang et al. 2025; Wermer et al. 2025). Nowadays, CBD use in sport horses is controversial, regulatory agencies like the Fédération Equestre Internationale (FEI) classifies CBD as a controlled substance (FEI 2025) and even traces in urine or blood can lead to penalties, making essential to distinguish pharmacologically active from irrelevant concentrations. With improvements in detection sensitivity, substances can be identified long after administration, even at levels with no physiological effect. To resolve this, a shift from exposure-based to effect-based monitoring has been proposed. The European Horseracing Scientific Liaison Committee (EHSLC) supports using pharmacokinetic/pharmacodynamic (PK/PD) models to determine "irrelevant" concentrations, as those unlikely to have any pharmacological impact (EHSLC 2012). These are known as Irrelevant Plasma Concentration (IPC) and Irrelevant Urinary Concentration (IUC) (EHSLC 2012). By integrating data on drug potency, bioavailability, therapeutic doses, and clearance, it is possible to calculate safe withdrawal intervals and detection times (DTs) to uniform doping control policies, as originally described by Toutain and Lassourd (2002), an approach that is widely applied today (Schenk et al. 2021; Minamijima et al. 2026).

Studies conducted in horses have shown that, following oral administration, CBD undergoes extensive first-pass hepatic metabolism, with the predominant formation of the metabolites 7-hydroxy-cannabidiol (7OH-CBD) and 7-carboxy-cannabidiol (7COOH-CBD) (Ryan et al. 2021; Sánchez de Medina et al. 2023). In particular, 7COOH-CBD represents the main inactive elimination metabolite, characterized by a long elimination half-life and prolonged detectability in biological fluids, which may complicate the distinction between pharmacologically relevant and irrelevant residues (Perucca and Bialer 2020; Ryan et al. 2021; Sánchez de Medina et al. 2023). As highlighted by Ryan et al. (2021), the persistence of this metabolite may lead to regulatory concerns, reinforcing the need to investigate metabolite kinetics in order to define reliable DTs (Ryan et al. 2021).

Efforts to define appropriate dosing regimens and withdrawal intervals are further hindered by the substantial interindividual variability in the pharmacokinetic profile of CBD in horses (Di Salvo et al. 2025), influenced by factors such as age, diet, stress, and gut microbiota (Cohen et al. 2021). Moreover, the low and variable oral bioavailability of CBD is largely attributed to poor aqueous solubility and extensive first-pass metabolism, which are factors largely influenced by the chemical and physical differences among the commercially available oral formulations (Perucca and Bialer 2020).

The present study aims to evaluate the pharmacokinetics of CBD and its major metabolites, 7COOH-CBD and 7OH-CBD, after a single oral dose of a water-soluble, Δ^9-tetrahydrocannabinol (THC)-free, formulation in healthy standardbred horses, to calculate pharmacologically irrelevant concentrations and estimate DTs by applying the IPC-IUC approach (Toutain and Lassourd 2002), contributing to fundamental knowledge to guide evidence-based recommendations for CBD use in equine athletes and support the development of rational withdrawal periods that balance therapeutic benefit with fair competition.

MATERIALS AND METHODS

Study design and sample collection

The study was conducted on six standardbred mares, aged 4 to 8 y.o. and weighing between 449±57.48kg. The study protocol was approved by the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_10_2023), and all horses were enrolled after obtaining written consent from the owners. A water-soluble, THC-free CBD formulation (KANARESCUE HORSES®, Ri.mos. (S.r.l.), Modena, Italy) was administered orally to all subjects as a single dose of 15mL, corresponding to 200mg of CBD.

Blood samples were collected via jugular venipuncture into tubes without anticoagulant to obtain plasma, while urine samples were obtained non-invasively through spontaneous urination. Sampling was performed at different time points before CBD administration (baseline, T0) and over a 120-hour period post-administration.

Chemicals and standards

All solvents and reagents used were Liquid Chromatography-Mass Spectrometry (LC-MS) grade and purchased from Merck (Darmstadt, Germany). Analytical standards for CBD, cannabiodiol-d3 (deuterium-labeled CBD-D_3), 7-COOH-CBD, 7-OH-CBD, and THC were obtained from Sigma-Aldrich (Round Rock, TX, USA).

Preparation of plasma samples

For compunds extraction from plasma, 0.5mL of equine plasma was transferred into a 15mL conic tube, then internal standard (10µL of CBD-D_3 at 1µg/mL in methanol) was added, followed by 1.5mL of tert-butyl methyl ether (TBME). Samples were vortexed for 1 min and centrifuged at 5500g for 10 min at 4°C. The organic phase was evaporated to dryness using a centrifugal evaporator. Residues were reconstituted in 100µL of methanol, vortexed for 30 seconds, transferred into 1.5mL Eppendorf tubes, and centrifuged at 15000g for 10min. The supernatants were then transferred into vials for LC coupled with high-resolution mass spectrometry (LC/HRMS) analysis.

Preparation of urine samples

A 1mL aliquot of urine was placed in a 15mL screw-cap tube and buffered with 1.5mL of 1M sodium acetate (pH 5.0). Enzymatic hydrolysis was performed using 10µL of β-glucuronidase from *Helix pomatia*, with incubation at 45°C for 4h. After cooling to room temperature, the mixture was transferred into a 15mL tube, and 20µL of CBD-D_3 (1 µg/mL in methanol) and 5mL of TBME were added. Following vortexing and centrifugation under the same conditions as the serum samples, the organic layer was evaporated to dryness. Samples were reconstituted in

100µL of methanol, vortexed, centrifuged and transferred to vials for LC/HRMS analysis.

Mass spectrometry analysis of CBD and its metabolites in equine plasma and urine

All samples were analyzed through LC/HRMS for the presence of CBD, 7COOH-CBD, 7OH-CBD, and THC using a Vanquish HPLC system (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Exploris high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

Chromatographic separation was achieved on a Raptor ARC-18 column (150 × 2.1 mm, 5 µm; Restek, Bellefonte, PA, USA) with a binary mobile phase of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A linear gradient from 98% A to 2% A over 15 min was applied at a flow rate of 0.3mL/min. The system was re-equilibrated after 21min, with a total run time of 25min. Column and autosampler temperatures were maintained at 30°C and 5°C, respectively.

Mass spectrometry was conducted using a heated electrospray ionization (HESI) source with a capillary temperature of 330°C and vaporizer temperature of 280°C. The electrospray voltage was set to 3.50 kV. Data acquisition was performed in full scan (FS) mode with a resolving power of 120,000 (scan range: m/z 70–800) and data-dependent MS2 (dd-MS2) mode for confirmation (resolving power: FS 60,000; dd-MS2 17,500). Fragmentation was optimized using a two-stage normalized collision energy (NCE) at 25 and 40 eV. Accurate mass, retention times, and fragment ions were used for compound identification and mass data of the studied compounds are reported in Table 1.

Method validation was performed following European guidelines (European Medicine Agency 2016) using pooled plasma and urine samples from untreated horses. Linearity was evaluated across five concentration points (0.05–50ng/mL) with correlation (r^2) values calculated from three calibration curves for each matrix. Specificity was confirmed by analyzing ten blank samples. Accuracy and precision were assessed at low (0.05ng/mL) and high (5ng/mL) levels for CBD, and 10ng/mL for its metabolites. Precision was expressed as intra- and inter-day coefficient of variation (CV%). The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios from blank samples: LOD = mean + 3×SD; LOQ = mean + 10×SD. Method validation parameters are reported in Table 2.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by using Phoenix WinNonlin software ver. 8.5 (Certara, Princeton, NJ, USA). All parameters were derived using non-compartmental analysis (NCA) models applied to the plasma concentration-time data.

To calculate IPC and IUC of CBD and its metabolites, the PK/PD approach developed by Toutain and Lassourd has been applied (Toutain and Lassourd 2002). This approach integrates pharmacokinetic data, such as plasma clearance and volume of distribution, with a conservative safety margin to establish thresholds that reliably identify concentrations with no pharmacological relevance. According to the model, the first step involves calculating the Effective Plasma Concentration (EPC), defined as the ratio of the therapeutic dose recommended by the manufacturer per dosing interval (τ; in this case 24 hours), to the plasma clearance (Cl). Mathematically, EPC is expressed as:

$$EPC = \frac{Dose \times \tau}{Clearance \times \tau}$$

Then, to determine the IPC, the EPC was divided by a Safety Factor (SF) set at 500 according to the most conservative approach (i.e. 10 x 50); the value 50 is normally used to transform the EPC into an ineffective concentration while the value 10 to account for interindividual variability. This yields:

$$IPC = \frac{EPC}{SF}$$

Table 1: Mass data of the compounds studied, including elemental compositions, polarities, theoretical mass-to-charge-ratio, exact mass-to-charge ratio, confirmation fragments of parent pseudomolecular ions and retention time (RT)

Analytes	Formula	Polarity	Theoretical mass-to-charge ratio (m/z)	Mass-to-charge-ratio (m/z)	Confirmation ions (m/z)	Retention Time (RT) (min)
CBD	$C_{21}H_{30}O_2$	+	315.23186	315.2310	193.22300	12.80
CBD-d3	$C_{21}H_{30}O_2$	+	318.25069	318.24925	195.06772	12.79
7-COOH CBD	$C_{21}H_{28}O_4$	-	343.19150	343.19146	299.20183	10.00
7-OH CBD	$C_{21}H_{30}O_3$	-	329.21220	329.21247	311.20154	10.18

Table 2: Method validation parameters for cannabidiol (CBD), 7-hydroxy-cannabidiol (7OH-CBD), and 7-carboxy-cannabidiol (7COOH-CBD)

Matrix	Calibration curve	r^2	LOD (ng/mL)	LOQ (ng/mL)	Spiked level (ng/mL)	Accuracy (%)	Precision CV (%)	
							intra-day	inter-day
CBD								
plasma	y = 0.0612x – 0.001	0.999	0.025	0.05	0.05	87	8.2	11.4
					5	92	4.5	9.2
urine	Y= 0.0576x + 0.002	0.994	0.05	0.1	0.05	94	9.2	13.4
					5	93	10.7	12.5
7COOH-CBD								
plasma	y = 0.3037x + 0.064	0.998	0.5	1	10	84	10	10.2
urine	y = 0.1749x - 0.166	0.997	0.5	1	10	80	8	8.3
7OH-CBD								
plasma	y = 0.0122x – 0.004	0.999	3	10	10	92	10	9.5
urine	y = 0.0053x – 0.01	0.993	3	10	10	80	8	7.6

The IUC was subsequently calculated by multiplying the IPC by the steady-state urine-to-plasma concentration ratio (R_{ss}), derived from the plasma and urine quantification data, thereby considering renal excretion dynamics:

$$IUC = IPC \times R_{ss}$$

To verify the conservativeness of these thresholds, the Residual Amount (RA) of the drug remaining in the body when plasma concentration reaches IPC was calculated as the product of IPC and the volume of distribution at pseudo-equilibrium (V_{area}) following a single dose administration of a drug:

$$RA = IPC \times V_{area}$$

Then, according to the selected approach, it was calculated in terms of percentage (%) of the administered therapeutic dose corresponding to the resulting RA. This value should be less than 1% of the administered dose to confirm the IPC and IUC values as both appropriate and regulatory compliant.

Finally, to check the reliability of IPC and IUC, the WT required to reach the IPC was calculated with the following equation:

$$WT = \frac{Log[\frac{Yz(X)}{D_x} \times (Cl \times \tau \times SF)]}{\lambda_z}$$

Where Yz represents the plasma intercept of the terminal slope λz determined following the administration of the therapeutic dose indicated by the manufacturer (D_x), Cl is the plasma clearance of the drug or its metabolite, τ represents the dosing interval (24 hours), and SF indicates the safety factor value of 500.

This stepwise process, while methodical, is fundamentally rooted in ensuring that plasma concentrations below the IPC are unlikely to exert any pharmacological effect, thus protecting both animal welfare and competition integrity (Toutain and Lassourd 2002).

RESULTS

Following the oral administration of a single 200mg dose of a water-soluble CBD formulation to each horse, the mean CBD dose administered in this study resulted 0.68±0.09 mg/kg. Plasma and urine concentrations of CBD, 7COOH-CBD, and 7OH-CBD were monitored in six standardbred mares over 120 hours. All animals successfully completed the study and regardless of group, none of the horses enrolled showed any signs of systemic adverse reactions. Importantly, no traces of THC or its metabolites were detected in any sample, confirming the THC-free status of the administered product.

In plasma samples the metabolite 7OH-CBD was never quantified: for this reason it was not possible to determine either its pharmacokinetic profile or its IPC-IUC calculation. Mean and standard deviation, were computed for the main pharmacokinetic parameters of both CBD and 7-COOH-CBD (Table 3). The results showed that CBD was characterized in plasma by rapid elimination and low systemic exposure (Fig. 1A). In contrast, its major metabolite 7COOH-CBD showed substantially long persistence and high exposure (Fig. 1B). However, both compounds displayed a marked interindividual variability (Fig. 1A and B).

Table 3: Pharmacokinetic parameters of cannabidiol (CBD) and 7-carboxy-cannabidiol (7COOH-CBD) after non-compartmental analysis

Parameter	Unit	Mean	S.D.
CBD			
Dose	mg/kg	0.68	0.09
Lambda_z	1/h	0.49	0.54
HL_lambda_z	h	2.52	1.54
T_{max}	h	1.5	0.84
C_{max}	ng/mL	1.98	1.29
AUC_{last}	h*ng/mL	4.77	2.34
V_{area}	mL/kg	474038.19	332749.21
Cl	mL/h/kg	151798.9	83393.47
MRT_{last}	h	2.68	1.16
7COOH-CBD			
Dose	mg/kg	0.68	0.09
Lambda_z	1/h	0.02	0.01
HL_lambda_z	h	32.47	9.51
T_{max}	h	6.5	3.21
C_{max}	ng/mL	67.24	40.67
AUC_{last}	h*ng/mL	2243.98	1384.99
V_{area}	mL/kg	17212.08	10314.4
Cl	mL/h/kg	358.38	183.61
MRT_{last}	h	39.36	6.96

S.D.: standard deviation; Lambda_z: elimination rate constant; HL_lambda_z: elimination half-life; T_{max}: time to reach maximum concentration; C_{max}: maximum concentration; AUC_{last}: area under the curve up to the last quantifiable concentration; V_{area}: volume of distribution at pseudo-equilibrium; Cl: clearance; MRT_{last}: mean residence time up to the last quantifiable concentration.

The complete plasma concentration-time profiles of CBD and 7COOH are reported in Fig. 1A and B, whereas urinary concentrations of CBD, 7COOH-CBD, and 7OH-CBD are reported in Fig. 2; due to the sampling scheme, it was not possible to perform the kinetic analysis of urinary data. As reported in Table 4, wich summarizes the results of the IPC and IUC calculations, short withdrawal times in plasma were calculated for CBD (1.2 days) compared with 7COOH-CBD (10.5 days).

Table 4: Irrelevant Plasma Concentration (IPC)-Irrelevant Urinary Concentration (IUC) calculation for cannabidiol (CBD) and 7-carboxy-cannabidiol (7COOH-CBD)

IPC-IUC CBD	
Effective Plasma Concentration (EPC)	0.19ng/mL
Irrelevant Plasma Concentration (IPC)	0.37pg/mL
Irrelevant Urine Concentration (IUC)	31.36pg/mL
Residual Amount (RA)	176.96ng/kg (0.026% of the dose)
Withdrawal Time to reach IPC (WT)	1.2 days
IPC-IUC 7COOH-CBD	
Effective Plasma Concentration (EPC)	79.14ng/mL
Irrelevant Plasma Concentration (IPC)	158.29pg/mL
Irrelevant Urine Concentration (IUC)	55.40pg/mL
Residual Amount (RA)	2724.43ng/kg (0.40% of the dose)
Withdrawal Time to reach IPC (WT)	10.5 days

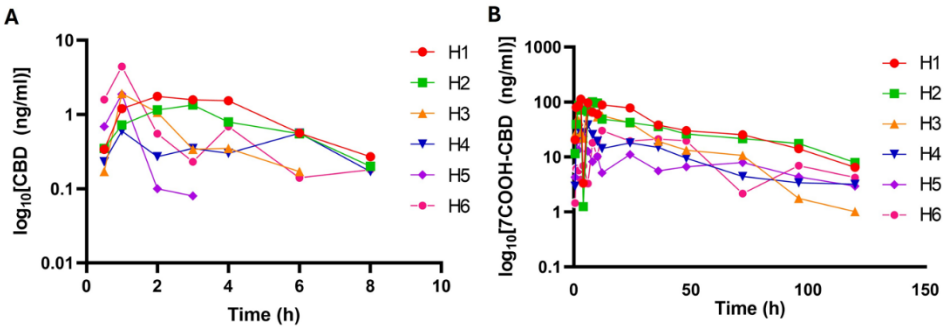

Fig. 1: A) Concentrations of CBD in plasma samples. B) Concentrations of 7COOH-CBD in plasma samples. Data are reported inng/mL.

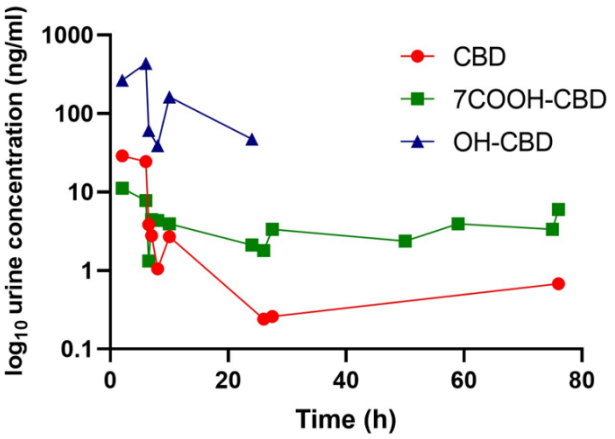

Fig. 2: Concentrations of CBD, 7COOH-CBD and 7OH-CBD in urine samples. Data are reported inng/mL.

DISCUSSION

This study provides foundational pharmacokinetic and safety data for CBD and its major metabolite 7COOH-CBD in sport horses, administered as a single oral dose of a water-soluble, THC-free formulation. The plasma concentration-time profiles, combined with the PK/PD modelling approach proposed by Toutain and Lassourd, enabled the calculation of IPC and IUC, offering regulatory guidance on WTs and DTs (Toutain and Lassourd 2002).

The pharmacokinetic profile of CBD in our study aligns with previous literature describing poor oral bioavailability and rapid elimination in equines (Ryan et al. 2021). Comparable findings were reported by Eichler et al. (2023), who observed rapid elimination and high variability in systemic exposure following oral CBD administration in horses (Eichler et al. 2023). Similarly, Williams et al. (2022) found low C_{max} and short half-life values (Williams et al. 2022). The results of the present study confirmed the short half-life (2.52±1.54h) and low C_{max} (1.98±1.29ng/mL) of CBD, reflecting rapid systemic clearance and high interindividual variability (Cl: 151798.9±83393.5mL/h/kg). These findings support the challenge of achieving and maintaining stable therapeutic plasma concentrations following oral administration and highlight the importance of formulation strategies to optimize CBD delivery in horses.

In contrast, 7COOH-CBD exhibited a markedly prolonged half-life (32.5±9.51h) and high systemic exposure (AUC_{last}: 2243.98±1384.99 h*ng/mL), consistent with its role as a major and persistent inactive metabolite.

From a regulatory perspective, the application of the Toutain and Lassourd's model allowed us to define effect-based thresholds. The calculated IPC for CBD (0.37pg/mL) and the corresponding IUC (31.36pg/mL) suggest that plasma concentrations below these values are unlikely to exert any pharmacological effect. Based on these values, the estimated WT for CBD to reach the IPC was calculated as approximately 1.2 days (29.79 hours), supporting a short elimination window in comparison to its metabolites. These thresholds provide an evidence-based alternative to the current detection-only approach, minimizing the risk of penalizing non-effective residual concentrations. Furthermore, the residual amount of CBD at IPC level was only 0.026% of the administered dose, well below the 1% threshold proposed by Toutain and Lassorud, confirming the robustness of the IPC-IUC values calculated in this study (Toutain and Lassourd 2002).

Similarly, the IPC for 7COOH-CBD (158.29pg/mL) and the associated IUC (55.40pg/mL) also fell within the threshold defined as not exerting any pharmacological activity, although the calculated WT resulted longer (10.5 days) due to its slow clearance. One implication of our findings is the prolonged detectability of 7COOH-CBD, which, despite being pharmacologically inactive, could lead to regulatory infractions under current doping control systems that do not distinguish between active and inactive residues. According to Toutain and Lassorud's approach, the establishment of WTs should be based on the most persistent substance, typically the metabolite with the longest elimination half-life. This strategy adopts a conservative stance by incorporating uncertainty factors to ensure regulatory safety margins (Toutain and Lassourd 2002; Lees et al. 2004). In this context 7COOH-CBD, although inactive (Ryan et al. 2021), represents a critical determinant for the overall withdrawal recommendation. This supports the necessity of including metabolite kinetics in DTs determinations, as emphasized by Ryan et al. (2021) and by the EHSLC (EHSLC 2012; Ryan et al. 2021). However, the lack of quantifiable 7OH-CBD in plasma and the high variability in pharmacokinetic parameters underscore the need for further investigation into formulation bioavailability, absorption kinetics, and metabolic pathways in equines. Factors such as diet, stress, gut microbiota, and interindividual variability may significantly influence cannabinoid metabolism and should be accounted for in future studies (Deabold et al. 2019).

This study presents some limitations that should be acknowledged. First, the animal sample size was enough and comparable to those of the other pharmacokinetic studies in equines (Ryan et al. 2021; Di Salvo et al. 2025), although due to the variability observed a larger number of animals would help in its clarification. Moreover, the study

was conducted under clinical conditions, which required urine collection through spontaneous urination: despite non-invasive, this approach partially hindered the definition of a full sampling scheme in the observation period, sometimes reducing the number and timing consistency of urine samples. Finally, potential sources of interindividual variability (e.g. sex, age, body condition, diet or gut microbiota) were not assessed, in line with the majority of published studies on oral CBD formulations in horses. In light of the increasing interest in CBD for use in horses, reflected by *in vitro* and *in vivo* studies on CBD effects are available (Ellis and Contino 2021; Turner et al. 2021b; St. Blanc et al. 2022; Turner et al. 2023; Battistin et al. 2025; Bazzano et al. 2025; Carroll et al. 2025), future studies should aim to expand the knowledge on its pharmacokinetics in this species, ideally through controlled experimental settings or randomized controlled clinical studies.

Conclusion

This study provides an evidence-based pharmacokinetic characterization of a water-soluble, THC-free CBD formulation and its major metabolite, 7COOH-CBD, in sport horses. Through the application of the Toutain and Lassourd PK/PD approach, IPC and IUC were defined for both compounds, enabling the calculation of WT. The results confirmed the rapid clearance and low systemic exposure of CBD following oral administration, contrasted by the prolonged persistence of 7COOH-CBD, which represents the primary determinant for withdrawal recommendations despite its presumed lack of pharmacological activity. Given the growing interest in the use of CBD in equine medicine for different therapeutic purposes, these findings highlight the importance of accounting for metabolite disposition within anti-doping control systems. Further studies are warranted to complete the pharmacokinetic characterization of CBD in horses and to clarify the actual pharmacological inactivity of its metabolites, thereby supporting regulatory decisions that safeguard both animal welfare and fair competition in equine athletes.

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Data Availability: The data presented in this study are available in the article and Supplementary Materials. Further information is available upon request from the Corresponding Author.

Ethics Statement: The study protocol was approved by the Institutional Ethics Committee for Animal Care of the University of Milan (approval n° OPBA_32_2022).

Author's Contribution: Conceptualization: Marco Montana and Petra Cagnardi; Methodology: Susanna Draghi and Federica Di Cesare; Software: Susanna Draghi and Petra Cagnardi; Validation: Susanna Draghi, Federica Di Cesare and Petra Cagnardi; Formal analysis: Federica Di Cesare and Petra Cagnardi; Investigation: Susanna Draghi, Federica Di Cesare, Linda Ucci, Francesco Ferrucci, Luca Stucchi, and Petra Cagnardi; Resources: Paola Catellani and Claudia Gemelli; Data curation: Susanna Draghi, Federica Di Cesare and Petra Cagnardi; Writing-original draft preparation: Susanna Draghi and Federica Di Cesare; Writing-review and editing: Federica Di Cesare, Marco Montana and Petra Cagnardi ; Visualization: Susanna Draghi, Francesco Arioli and Petra Cagnardi; Supervision: Petra Cagnardi; Project administration: Petra Cagnardi; Funding acquisition: Francesco Ferrucci and Petra Cagnardi. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

Battistin L, Moya LF, Ferreira LV, Braz AM, Carvalho MD, Golim MD and Amorim RM, 2025. In vitro immunomodulatory effects of equine adipose tissue-derived mesenchymal stem cells primed with a cannabidiol-rich extract. International Journal of Molecular Sciences 26(9): 4208. https://doi.org/10.3390/ijms26094208

Bazzano M, Laus F, Cerquetella M, Spaterna A and Marchegiani A, 2025. Effect of orally administered cannabidiol oil on daily tonometric curve in healthy Italian Saddle horses. PloS one 20(5): e0325191. https://doi.org/10.1371/journal.pone.0325191

Bombardi C, Salamanca G, Tagliavia C, Grandis A, Zamith Cunha R, Gramenzi A, De Silva M, Zannoni A and Chiocchetti R, 2025. Cannabinoid receptors in the horse lateral nucleus of the amygdala: a potential target for ameliorating pain perception, stress and anxiety in horses. International Journal of Molecular Sciences 26(15): 7613. https://doi.org/10.3390/ijms26157613

Carroll AT, Reed RA, Berghaus LJ, McNabney D and Knych HK, 2025. Oral cannabidiol increases thermal threshold in horses without physiologic adverse effects. American Journal of Veterinary Research 86(11). https://doi.org/10.2460/ajvr.25.05.0185

Cohen L, Jones T, Guay K, Smith WB, Nichols J and Elwonger F, 2021. 62 Evaluation of oral supplementation of cannabidiol (CBD) in horses. Journal of Equine Veterinary Science 100: 103525. https://doi.org/10.1016/j.jevs.2021.103525

Deabold KA, Schwark WS, Wolf L and Wakshlag JJ, 2019. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. Animals 9(10): 832. https://doi.org/10.3390/ani9100832

Di Salvo A, Bazzano M, Rocca GD, Galarini R, Marchegiani A, Paoletti F, Giusepponi D, Mantovani M and Laus F, 2025. Pharmacokinetics of a single oral administration of two cannabidiol formulations in fed and fasted horses. Frontiers in Veterinary Science. 12: 1515833. https://doi.org/10.3389/fvets.2025.1515833

Draeger AL, Thomas EP, Jones KA, Davis AJ and Porr CS, 2021. The effects of pelleted cannabidiol supplementation on heart rate and reaction scores in horses. Journal of Veterinary Behavior 46: 97-100. https://doi.org/10.1016/j.jveb.2021.09.003

Eichler F, Poźniak B, Machnik M, Schenk I, Wingender A, Baudisch N, Thevis M, Bäumer W, Lischer C and Ehrle A, 2023. Pharmacokinetic modelling of orally administered cannabidiol and implications for medication control in horses. Frontiers in Veterinary Science 10: 1234551. https://doi.org/10.3389/fvets.2023.1234551

Ellis KL and Contino EK, 2021. Treatment using cannabidiol in a horse with mechanical allodynia. Equine Veterinary Education 33(4): e79-82. https://doi.org/10.1111/eve.13168

European Horserace Scientific Liason Committee (EHSLC) 2012. The policies of European Horserace Scientific Liaison Committee. Retrieved 07/22, 2025, from https://www.ehslc.com/

European Medicine Agency, 2016. VICH GL49: Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Validation of analytical methods used in residue depletion studies. Retrieved 07/22, 2025, from https://www.ema.europa.eu/en/vich-gl49-studies-evaluate-metabolism-residue-kinetics-veterinary-drugs-food-producing-animals-validation-analytical-methods-used-residue-depletion-studies-scientific-guideline

Fédération Équestre Internationale (FEI) 2025. FEI Clean Sport – Prohibited Substances Database. Retrieved 07/22, 2025, from https://prohibitedsubstancesdatabase.feicleansport.org/

Kogan LR, Hellyer PW, Silcox S and Schoenfeld-Tacher R, 2019. Canadian dog owners' use and perceptions of cannabis products. The Canadian Veterinary Journal 60(7): 749.

Kupczyk P, Rykala M, Serek P, Pawlak A, Slowikowski B, Holysz M, Chodaczek G, Madej JP, Ziolkowski P and Niedzwiedz A, 2022. The cannabinoid receptors system in horses: Tissue distribution and cellular identification in skin. Journal of Veterinary Internal Medicine 36(4): 1508-24. https://doi.org/10.1111/jvim.16467

Lees P, Landoni MF, Giraudel J and Toutain PL, 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. Journal of Veterinary Pharmacology and Therapeutics 27(6): 479-90. https://doi.org/10.1111/j.1365-2885.2004.00617.x

Luedke C and Wilhelm T, 2021. Cannabinoids in Equine Medicine. InCannabis Therapy in Veterinary Medicine: A Complete Guide. (pp. 295-305). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-68317-7_12

Miagkoff L, Girard CA, St-Jean G, Richard H, Beauchamp G and Laverty S, 2023. Cannabinoid receptors are expressed in equine synovium and upregulated with synovitis. Equine Veterinary Journal 55(4): 681-95. https://doi.org/10.1111/evj.13860

Minamijima Y, Kuroda T, Okano A, Wakuno A, Yuasa R, Ishikawa Y, Nomura M, Kinoshita K and Yamada M, 2026. Plasma and urine pharmacokinetics of intravenous pridinol in thoroughbreds for its medication control. Journal of Veterinary Pharmacology and Therapeutics 49(1): 1-6. https://doi.org/10.1111/jvp.70029

Perucca E and Bialer M, 2020. Critical aspects affecting cannabidiol oral bioavailability and metabolic elimination, and related clinical implications. CNS Drugs 34(8): 795-800. https://doi.org/10.1007/s40263-020-00741-5

Ryan D, McKemie DS, Kass PH, Puschner B and Knych HK, 2021. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. Drug Testing and Analysis 13(7): 1305-17. https://doi.org/10.1002/dta.3028

Sánchez de Medina A, Serrano-Rodríguez JM, Díez de Castro E, García-Valverde MT, Saitua A, Becero M, Muñoz A, Ferreiro-Vera C and Sánchez de Medina V, 2023. Pharmacokinetics and oral bioavailability of cannabidiol in horses after intravenous and oral administration with oil and micellar formulations. Equine Veterinary Journal 55(6): 1094-103. https://doi.org/10.1111/evj.13923

Schenk I, Machnik M, Broussou D, Meuly A, Roques BB, Lallemand E, Düe M, Röttgen H, Lagershausen H, Toutain PL and Thevis M, 2021. Kinetic disposition of diazepam and its metabolites after intravenous administration of diazepam in the horse: Relevance for doping control. Journal of Veterinary Pharmacology and Therapeutics 44(5): 733-44. https://doi.org/10.1111/jvp.12991

St. Blanc MP, Chapman AM, Keowen ML, Garza Jr F, Liu CC, Gray L and Andrews FM, 2022. Effects of a supplement containing cannabidiol (CBD) on sedation and ataxia scores and health. Journal of Equine Veterinary Science 117: 104085. https://doi.org/10.1016/j.jevs.2022.104085

Temmerman R, 2025. Editorial: Use of cannabis derivatives in veterinary medicine. Frontiers in Veterinary Science 12: 1539422. https://doi.org/10.3389/fvets.2025.1539422

Toutain PL and Lassourd V, 2002. Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse. Equine Veterinary Journal 34(3): 242–249. https://doi.org/10.2746/042516402776185985

Trevisiol S, Popot MA, Garcia P, Boyer S, Caroff M, Drif L, Taleb W, Tendon S, Moulard Y and Bailly-Chouriberry L, 2025. In vivo comparative study of hemp straw exposure and cannabidiol oil administration in horse urine. Drug Testing and Analysis 17(6): 805-11. https://doi.org/10.1002/dta.3783

Turner PL, Guay KA, Jones T, Cohen L, Elwonger F, Van Geem R and Smith WB, 2021a. The effects of feeding cannabidiol oil on behavior of stalled horses. Journal of Animal Science 99(Supplement_3): 5. https://doi.org/10.1093/jas/skab235.008

Turner S, Barker VD and Adams AA, 2021b. Effects of cannabidiol on the in vitro lymphocyte pro-inflammatory cytokine production of senior horses. Journal of Equine Veterinary Science 103:103668. https://doi.org/10.1016/j.jevs.2021.103668

Turner S, Knych HK and Adams AA, 2023. The effects of cannabidiol on immune function and health parameters in senior horses. Veterinary Immunology and Immunopathology 257: 110549. https://doi.org/10.1016/j.vetimm.2023.110549

Ukai M, McGrath S and Wakshlag J, 2023. The clinical use of cannabidiol and cannabidiolic acid–rich hemp in veterinary medicine and lessons from human medicine. Journal of the American Veterinary Medical Association 261(5): 623-31. https://doi.org/10.2460/javma.23.02.0064

Wang TC, Wakshlag JJ, Jager MC, Schwark WS, Trottier NL, Chevalier JM, Pearson G and Cercone M, 2025. Chronic oral dosing of cannabidiol and cannabidiolic acid full-spectrum hemp oil extracts has no adverse effects in horses: a pharmacokinetic and safety study. American Journal of Veterinary Research 86(3). https://doi.org/10.2460/ajvr.24.08.0235

Wermer K, Korbacska-Kutasi O, Berkecz R, Csupor D, Ágh N, Sztojkov-Ivanov A and Cserhalmi D, 2025. Pharmacokinetics of cannabidiol and its two main phase I metabolites in Connemara ponies. Frontiers in Veterinary Science 12: 1599934.

https://doi.org/10.3389/fvets.2025.1599934

Williams MR, Holbrook TC, Maxwell L, Croft CH, Ientile MM and Cliburn K, 2022. Pharmacokinetic evaluation of a cannabidiol supplement in horses. Journal of Equine Veterinary Science 110: 103842. https://doi.org/10.1016/j.jevs.2021.103842

Yocom AF, O'Fallon ES, Gustafson DL and Contino EK, 2022. Pharmacokinetics, safety, and synovial fluid concentrations of single-and multiple-dose oral administration of 1 and 3 mg/kg cannabidiol in horses. Journal of Equine Veterinary Science113: 103933. https://doi.org/10.1016/j.jevs.2022.103933

Zamith Cunha R, Gobbo F, Morini M, Zannoni A, Mainardi C, D'arpe L, Gramenzi A and Chiocchetti R, 2025. Distribution of endocannabinoid system receptors in the equine hoof: dysregulation as a potential therapeutic target for laminitis. Histochemistry and Cell Biology 163(1): 71. https://doi.org/10.1007/s00418-025-02397-y

Zamith Cunha R, Semprini A, Salamanca G, Gobbo F, Morini M, Pickles KJ, Roberts V and Chiocchetti R, 2023a. Expression of cannabinoid receptors in the trigeminal ganglion of the horse. International Journal of Molecular Sciences 24(21): 15949. https://doi.org/10.3390/ijms242115949

Zamith Cunha R, Zannoni A, Salamanca G, De Silva M, Rinnovati R, Gramenzi A, Forni M and Chiocchetti R, 2023b**.** Expression of cannabinoid (CB1 and CB2) and cannabinoid-related receptors (TRPV1, GPR55, and PPARα) in the synovial membrane of the horse metacarpophalangeal joint. Frontiers in Veterinary Science 10: 1045030. https://doi.org/10.3389/fvets.2023.1045030