

Effects of vitamins A and E on eprinomectin transport in Caco-2 cells

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Abstract: Eprinomectin (EPR) is a macrocyclic lactone antiparasitic widely used in veterinary medicine. Although the off-label oral use of the pour-on formulation of EPR is known to be effective and practical, data regarding its interactions with foods or vitamin supplements that may affect intestinal absorption remain limited. This study investigated the effects of vitamin A (VA) and vitamin E (VE) on the transepithelial permeability of EPR using the Caco-2 cell monolayer model. An analytical method for EPR quantification was developed using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The integrity of tight junction dynamics in epithelial monolayers was monitored using transepithelial electrical resistance (TEER) throughout the 21-day differentiation protocol. Apparent permeability coefficients (P_{app}) were calculated in both apical-to-basolateral and basolateral-to-apical directions and compared between EPR alone and EPR combined with the vitamins. EPR alone exhibited moderate permeability across Caco-2 monolayers ($7.64 \pm 2.27 \times 10^{-6}$ cm/s). Notably, our results demonstrate that co-treatment of EPR with VA alone, as well as co-treatment with the combination of VA and VE, significantly reduced EPR permeability in vitro ($P < 0.001$). These findings highlight possible food/nutrient-drug interactions relevant to veterinary oral drug administration, and additional mechanistic and *in vivo* studies will be necessary to clarify the underlying pathways.

Keywords: Caco-2 transwell model; eprinomectin; fat-soluble vitamins; intestinal permeability; P-glycoprotein

Anthelmintic macrocyclic lactones (MLs) are antiparasitic drugs with broad-spectrum activity, favourable safety profiles, and ease of administration (Campbell 2012; Geary and Moreno 2012;

Mohammed and Ahmed 2024), and are widely used in both animals (McKellar and Gokbulut 2012) and humans (Molyneux et al. 2003). Eprinomectin [4'-epiacetylamino-4''deoxyavermectin B1], a mem-

ber of the avermectin class of MLs, is used for the treatment of internal and external parasites in animals such as cattle, sheep, goats, camels, pigs, rabbits, and dogs (Figure 1) (EMA 1996; Hinney et al. 2022). Binding of eprinomectin (EPR) to glutamate-gated chloride channels increases chloride conductance across the cell membrane, resulting in hyperpolarisation and flaccid paralysis of parasites (Stern et al. 2025).

EPR has been approved as both a pour-on and a subcutaneous (S/C) injectable formulation in several countries and provides high therapeutic efficacy against a broad range of gastrointestinal nematodes and *Dictyocaulus filaria* lungworms in target animal species (Rehbein et al. 2014; Hamel et al. 2018). Several studies have shown that the pour-on administration of EPR has some limitations under field conditions (Murri et al. 2014; Rambozzi et al. 2025). In this context, several challenges have been documented, including the reduced bioavailability of EPR observed in lactating goats compared with dry goats, the need for higher dose rates in goats than in cattle (specifically 1.0 mg/kg b.w. instead of 0.5 mg/kg b.w.), and field observations suggesting inconsistent efficacy of topical EPR as assessed by post-treatment faecal egg count (FEC) reductions (Lespine et al. 2012; Murri et al. 2014; Bordes et al. 2022). On the other hand, it has been reported that oral administration of the pour-on formulation of EPR is a practical method of administration in goats (Rostang et al. 2020).

Although numerous studies have primarily emphasised the pour-on and S/C pharmacokinetic profiles of EPR in various animal species (Gokbulut et al. 2011; Rehbein et al. 2014; Miller et al. 2025), particularly for off-label use, pharmacokinetic data

remain limited. Badie et al. (2015) determined that oral administration of the topical EPR formulation (0.5 or 1.0 mg/kg b.w.) to goats led to faster absorption and significantly higher peak concentrations (C_{max}) in plasma and milk compared to the traditional pour-on route. This increased systemic availability was linked to high anthelmintic efficacy, demonstrating 100% effectiveness against *H. contortus* and 99.8% against *T. colubriformis* at the 0.5 mg/kg dose. To evaluate the potential of an orally administered drug as a rational therapeutic option, it is essential to fully characterise the influence of the nutritional status (fasted versus fed conditions) on its systemic exposure and drug delivery mechanisms (CDER 2002; DeHaven and Conner 2014). Fat-soluble vitamins, such as vitamins A and E, are routinely and widely used as nutritional additives in animal feeding (EFSA 2010; EFSA 2013). Certain fat-soluble vitamins have been reported to modulate the absorption of P-gp substrate drugs through the induction or inhibition of transmembrane transporter proteins, particularly P-gp and BCRP (Thomas 1995; Podszun and Frank 2014). These findings suggest that intestinal absorption is a key determinant of EPR systemic exposure when orally administered.

Recent studies have shown that some fat-soluble vitamins (A and E) can modulate the intestinal absorption of some drugs by interacting with P-gp, which may limit the absorption of orally administered drugs (Lespine et al. 2009; Husain et al. 2022). For instance, D- α -tocopheryl polyethylene glycol succinate (TPGS), an FDA-approved pharmaceutical adjuvant, is recognised as a potent P-gp inhibitor that enhances the intracellular accumulation of substrates (Yang et al. 2018; Husain et al. 2022). Similarly, vitamin A palmitate (VA palmitate), a GRAS compound, has been confirmed to inhibit the P-gp transporter; it significantly increased the permeability of P-gp substrates (Reker et al. 2020). In addition to transporter-mediated effects, fat-soluble vitamins may also influence drug permeability through physicochemical interactions at the intestinal epithelial membrane. EPR, as a lipophilic compound, is particularly susceptible to such interactions (Lespine et al. 2009). Due to their lipophilic nature, vitamins A and E can partition into the lipid bilayer, where they may alter membrane fluidity and lipid organisation and potentially sequester lipophilic drug molecules within the membrane, thereby influencing their availability and diffusion

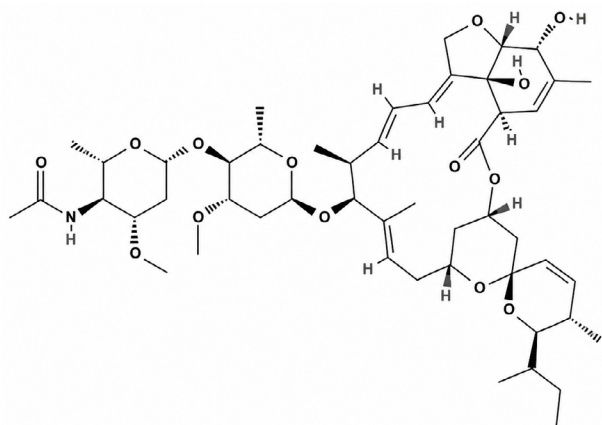


Figure 1. Molecular structure of eprinomectin

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at the apical interface (Atkinson et al. 2021; Radzin et al. 2023). These membrane-level interactions may affect transcellular diffusion independently of classical efflux transporter modulation (Wang and Quinn 2000). Furthermore, fat-soluble vitamins may influence P-gp activity through both direct and indirect mechanisms. Direct effects may include inhibition of P-gp ATPase activity or competition at substrate-binding sites, whereas indirect effects may arise from alterations in membrane fluidity and lipid microenvironment that can modify P-gp conformation and function. Additionally, fat-soluble vitamins may regulate P-gp expression via nuclear receptor-mediated pathways, such as the pregnane X receptor (PXR), thereby affecting the transport of P-gp substrates such as EPR (Lespine et al. 2009; Husain et al. 2022).

Supporting the relevance of nutrient–drug interactions involving macrocyclic lactones, an *in vivo* study confirmed that high oral doses of vitamins A and E, as well as their combination, significantly increased the plasma and brain concentrations of ivermectin, a well-established P-gp substrate, thereby suggesting a transporter-mediated modulation of drug disposition (Tras et al. 2023). Such vitamin-mediated modulation of P-gp highlights the relevance of investigating whether similar mechanisms affect the intestinal transport of EPR. Beyond formulation- and species-related factors, therefore, intestinal transport mechanisms play a critical role in determining the systemic availability of orally administered macrocyclic lactones. In this context, EPR is a known substrate of ATP-binding cassette transporters, particularly P-gp, which limits transepithelial drug absorption at the intestinal epithelium (Kiki-Mvouaka et al. 2010).

Despite its widespread use, the interactions of EPR with vitamins during transepithelial transport have not been fully elucidated. Our study aimed to investigate the effects of VA and VE, two of the most frequently supplemented micronutrients in livestock and horses (McDowell 2006; Verhoeckx et al. 2015), on the transepithelial transport of EPR. To this end, the Caco-2 cell monolayer model, which is widely recognised for its close correlation with *in vivo* intestinal absorption and for its expression of major efflux and uptake transporters, was employed to assess EPR permeability. Subsequently, EPR concentrations were quantified using a validated UPLC-MS/MS method to ensure

sensitive and accurate analytical evaluation. To the best of our knowledge, this is the first study to systematically evaluate how fat-soluble vitamins affect the intestinal permeability of EPR.

MATERIAL AND METHODS

Reagents and drugs

EPR reference standard (a mixture of component B1a ~95% and component B1b ~2.6%) (USP, Rockville, MD, USA), vitamin A propionate, and vitamin E were kindly donated by Ceva Animal Health (İstanbul, Türkiye). Dulbecco's modified Eagle's medium (DMEM) was obtained from Sigma Aldrich (St. Louis, MO, USA). Caco-2 cell line was supplied by the American Type Culture Collection (ATCC) (Manassas, VA, USA). Foetal bovine serum, penicillin/streptomycin solution (100×), trypsin (0.25%)-EDTA (0.02%) solution, and Hank's balanced salt solution (HBSS) were obtained from Biochrom AG (Berlin, Germany). Lucifer yellow CH was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). All other compounds used were commercially available reagent-grade chemicals.

Cell culture

The permeability of EPR in combination with VA and VE was evaluated using the human colorectal adenocarcinoma cell line (Caco-2). Caco-2 cells between passages 35 and 42 were cultured at 37 °C in a humidified atmosphere containing 5% CO₂. The cell culture medium contained DMEM supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% penicillin/streptomycin solution (100 IU/ml penicillin and 100 µg/ml streptomycin). Upon reaching 80–85% confluence within 4–7 days, cells were detached using trypsin–EDTA and subsequently seeded onto polycarbonate membrane inserts (1 µm pore size, 12-well cell culture plate; Greiner Bio-One, Frickenhausen, Germany) at a density of 50 000 cells per well. The cells were cultured for 21–22 days to allow for monolayer formation. The culture medium was refreshed every other day, and monolayer integrity was monitored by measuring transepithelial electrical resistance (TEER) using a Millicell-ERS volt-ohm meter (Merck Millipore, Darmstadt, Germany).

Permeability assay

Permeability studies were conducted using Caco-2 cells to evaluate the permeability of EPR alone and in combination with VA and VE. Metoprolol tartrate and lucifer yellow were used as positive and negative controls, respectively, to confirm monolayer integrity in accordance with ICH guidelines (ICH 2019). Cells were seeded onto 12-well Transwell® inserts (pore size: 1 µm; surface area: 113.1 mm²). A total of 500 µl of cell suspension was added to the apical compartment, while 1 ml of fresh culture medium was added to the basolateral compartment. For the initial three days, the plates were maintained in an incubator (37 °C, 5% CO₂) without any treatment to allow the cells to grow. On the following days, the culture medium was replaced with fresh medium every other day. On day 21, TEER values were measured to assess monolayer

integrity. Only monolayers exhibiting TEER values between 400 and 600 Ω·cm² were selected for the assay (Artursson and Karlsson 1991). Drug-free HBSS was added to both compartments and the cells were then incubated for 30 minutes. All permeability experiments were performed under serum-free conditions using HBSS. EPR (20 µg/ml) and its combinations with VA (33 µM) and VE (33 µM) were prepared in HBSS (Collnot et al. 2006). For the permeability test, 0.5 ml of the test solution was added to the apical compartment, and 1 ml of HBSS (drug-free) was added to the basolateral compartment. The inserts were incubated for 2 h at 37 °C (Figure 2). Following incubation, samples were collected and analysed by UPLC-MS/MS to quantify the amount of drug transported to the basolateral side. Apparent permeability coefficients (P_{app} , cm/s) for EPR, EPR + VA, EPR + VE, and EPR + VA + VE were calculated using Equation 1.

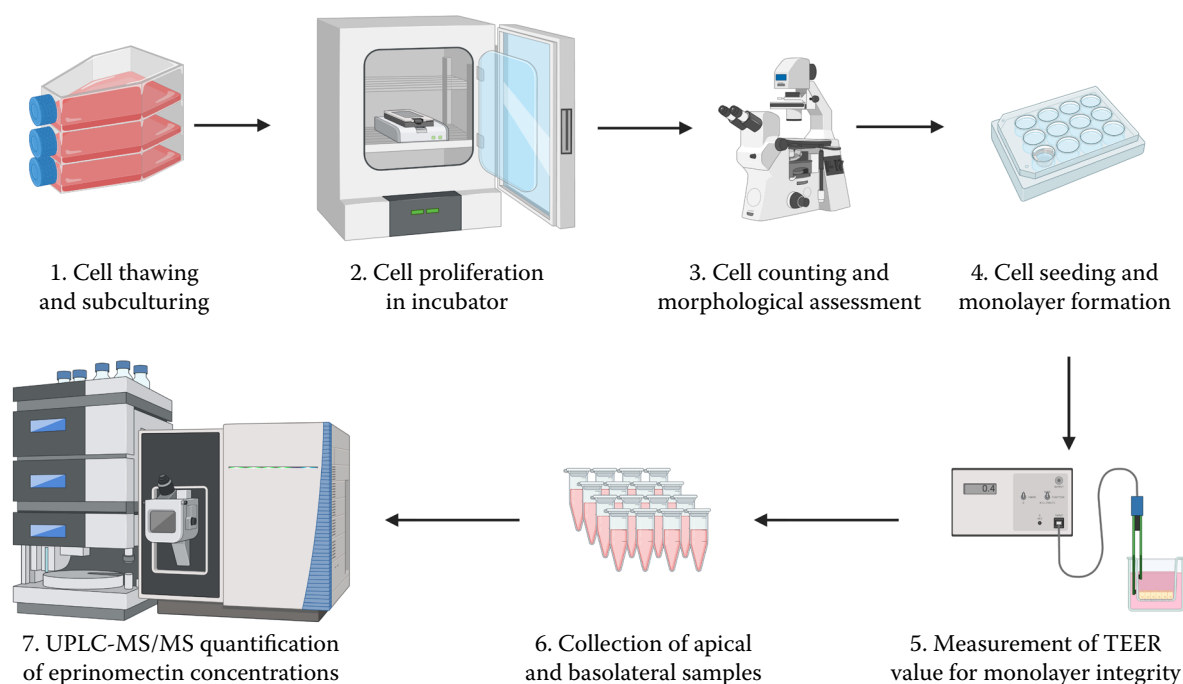


Figure 2. Schematic representation of the Caco-2 cell permeability assay for eprinomectin

1. Cell thawing and subculturing: Frozen Caco-2 cells were thawed and expanded under sterile conditions. 2. Cell proliferation in an incubator: Cells were maintained under standard culture conditions (37 °C, 5 % CO₂, humidified atmosphere). 3. Cell counting and morphological assessment: Cell density and morphology were evaluated microscopically before seeding. 4. Cell seeding and monolayer formation: Cells were seeded onto permeable inserts and cultured until a confluent monolayer was formed. 5. Measurement of TEER values for monolayer integrity: Transepithelial electrical resistance (TEER) was measured to verify tight-junction integrity prior to the permeability assay. 6. Collection of apical and basolateral samples: After incubation with eprinomectin and/or vitamin treatments, samples were collected from both compartments. 7. UPLC-MS/MS quantification of eprinomectin concentrations: Eprinomectin concentrations in the collected samples were determined by liquid chromatography coupled with tandem mass spectrometry. Created in <https://BioRender.com>

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$$P_{\text{app}} = [dQ/dt] \times [1/(A \times C_0)] \quad (1)$$

where:

dQ/dt – cumulative transport rate ($\mu\text{M}/\text{min}$);

A – surface area of inserts (cell monolayer) (cm^2);

C_0 – initial concentration in the apical region (μM).

Sample preparation and UPLC-MS/MS analysis

To determine the transport rate of the test compound, an extraction procedure was performed prior to sample injection into the UPLC-MS/MS system. Calibration standards and quality control samples were prepared by spiking blank buffer. Subsequently, 50 μl of blank buffer was dispensed into two vials labelled as “blank” and “blank + IS” (internal standard). Then, 50 μl of the corresponding sample was transferred to the vials, followed by vortex mixing.

A volume of 0.15 ml of selamectin was added to all samples as the IS, and the mixtures were placed on a shaker for 5 minutes. The samples were centrifuged at $1\,800 \times g$ for 5 min at 20°C . The supernatants were transferred into autosampler vials, and 5 μl of each was injected into the UPLC-MS/MS system (Figure 2).

The qualitative and quantitative determination of EPR was carried out using a Dionex Ultimate 3000 UPLC system coupled with a TSQ Fortis (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an ESI source operating in positive ionisation mode. The chromatographic separation was carried out using a Raptor Inert C18 column ($50 \times 2.1\text{ mm}$, $2.7\text{ }\mu\text{m}$; Restek, Bellefonte, PA, USA) with solvent A (5 mM ammonium formate + 0.1% formic acid) and solvent B (methanol + 0.1% formic acid). The following gradient was applied: 0–1.0 min, 5% B; 1.0–3.0 min, 5–90% B; 3.0–6.0 min, 90% B; and 6.0–8.0 min, 5% B for column re-equilibration. The flow rate, injection volume, and column temperature were 0.4 ml/min, 5 μl , and 40°C , respectively. HESI interface conditions with multiple reaction monitoring (MRM) modes were spray voltage, static; sheath gas pressure, 45 arb; aux gas pressure, 15 arb; capillary temperature, 300°C ; and vaporiser temperature, 350°C . Data were processed using TraceFinder v3.2 software (Thermo Fisher Scientific, Waltham, MA, USA).

Calibration and method validation

Method validation was carried out by constructing 18 independent calibration curves, each demonstrating linearity across the tested concentration range. The overall variance was assessed by calculating the relative deviation of each standard point from the regression curve. Standard deviations were less than 10% throughout the calibration range for EPR. At the LOQ (limit of quantification), accuracy (70–120%) and precision (CVs $\leq 20\%$) were acceptable. In addition to LOQ evaluation, six replicate measurements were performed for six concentration levels (0.1, 0.25, 0.5, 1, 5, and 10 $\mu\text{g}/\text{ml}$). An accuracy range of 85–96% was obtained, and CVs of 25% under repeatability conditions were used to assess precision. Under reproducibility conditions, the LOQ accuracy of three fortified samples varied from 70% to 120%, with CVs of 15%. The limit of detection (LOD) and LOQ values for EPR were determined by the UPLC-MS/MS system and are presented in Table 1.

Statistical analysis

Results are expressed as the mean $\pm$ SD. The significance of differences in the P_{app} values was determined by one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test.

Table 1. Validation parameters of the analytical method for eprinomectin quantification

Parameters	EPR chromatographic conditions
Linearity range ($\mu\text{g}/\text{ml}$)	10–0.1
R^2 value	0.993 6
LOD (ng/ml)	0.32
LOQ (ng/ml)	0.98
Intraday repeatability (%CV); $n = 6$	3.8
Interday repeatability (%CV); $n = 6$	7.4
Recovery (5 $\mu\text{g}/\text{ml}$, 0.5 $\mu\text{g}/\text{ml}$, 0.1 $\mu\text{g}/\text{ml}$)	96%, 94%, 85%
Retention time (min)	5.86

High recovery was obtained with the UPLC-MS/MS method; the R^2 value was close to 1; the limits of detection and limits of quantification were low, and intraday to interday repeatability was $<10\%$

CV = coefficient of variation; LOD = limit of detection; LOQ = limit of quantification

RESULTS

Permeability studies were conducted using Caco-2 cell monolayers to assess the transcellular transport of EPR (Figure 2). According to the results presented in Table 2 and Figure 3, the apparent permeability coefficients (P_{app}) of EPR alone, EPR + VA, EPR + VE, EPR + VA + VE, and the control compounds were calculated.

Metoprolol exhibited high permeability ($88.49 \pm 1.42 \times 10^{-6}$ cm/s), whereas Lucifer yellow demonstrated low permeability ($2.49 \pm 1.03 \times 10^{-6}$ cm/s) (Figure 3). EPR alone exhibited a mean P_{app} value of $7.64 \pm 2.27 \times 10^{-6}$ cm/s, indicating moderate permeability across Caco-2 monolayers. Co-treatment with VA significantly reduced EPR permeability to $4.58 \pm 0.52 \times 10^{-6}$ cm/s ($P < 0.001$). The combination of VA and VE also markedly reduced EPR permeability ($5.06 \pm 0.56 \times 10^{-6}$ cm/s, $P < 0.001$). Although a reduction in EPR permeability was seen with VE alone ($6.41 \pm 0.93 \times 10^{-6}$ cm/s), the difference was not statistically significant. The analytical method applied, validated in accordance with current international guidelines (ICH 1996) (Table 1), enabled the selective and accurate identification and quantification of EPR. The chromatographic separation exhibited no overlap or interference at the retention time of EPR from matrix components or degradation products. Furthermore, no degradation products of EPR were detected using UPLC-MS/MS analysis at the end of the experiments.

Table 2. Transport parameters of eprinomectin across Caco-2 transwell model

Drug/ formulation	P_{app} (10^{-6} cm/s)		
	EPR	Metoprolol	Lucifer yellow
EPR	7.64 ± 2.27	–	–
EPR + VA	$4.58 \pm 0.52^{***}$	–	–
EPR + VE	6.41 ± 0.93	–	–
EPR + VA + VE	$5.06 \pm 0.56^{***}$	–	–
Metoprolol	–	88.49 ± 1.42	–
Lucifer yellow	–	–	2.49 ± 1.03

Results were expressed as mean $\pm$ SD of three independent experiments in triplicate ($n = 3$). A significant decrease in P_{app} was observed with the addition of vitamin A alone and in combination with vitamin E ($^{***}P < 0.001$ vs EPR alone). Metoprolol and Lucifer yellow were used as positive and negative controls for transport, respectively

EPR = eprinomectin; VA = vitamin A; VE = vitamin E

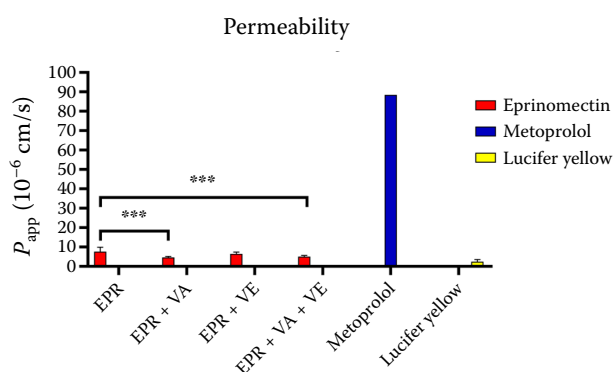


Figure 3. Apparent permeability coefficients (P_{app} , $\times 10^{-6}$ cm/s) of eprinomectin (EPR) and its combinations with fat-soluble vitamins across Caco-2 cell monolayers. Statistical significance was evaluated using one-way ANOVA followed by Tukey's multiple comparison test ($^{***}P < 0.001$). Data are expressed as mean $\pm$ SD ($n = 3$)

Treatments included eprinomectin alone (EPR), eprinomectin with vitamin A (EPR + VA), eprinomectin with vitamin E (EPR + VE), and eprinomectin with both vitamins A and E (EPR + VA + VE). Metoprolol and Lucifer yellow were used as high- and low-permeability reference compounds, respectively

DISCUSSION

The reliability of the Caco-2 permeability assay was assessed through validation steps that confirmed the integrity of the monolayer and the suitability of the model. Monolayer integrity was controlled by ensuring TEER values of $>300 \Omega \cdot \text{cm}^2$. Metoprolol, a high-permeability marker, exhibited significantly higher P_{app} values than EPR, while Lucifer yellow demonstrated limited paracellular transport, affirming monolayer integrity (Hidalgo et al. 1989; ICH 2019). These findings are consistent with previously reported data in the literature (Lennernas et al. 1996; Koljonen et al. 2006). Consequently, this indicates that the Caco-2 monolayers formed an intact and uniform monolayer epithelial barrier that effectively mimics the intestinal epithelium, thereby validating their reliability for use in *in vitro* permeability studies. LC-MS/MS analysis was performed on samples collected from both the apical and basolateral sides in the transport experiments to determine the permeability of EPR across the Caco-2 cell monolayer. The inherent high specificity and sensitivity of UPLC-MS/MS provided greater analytical accuracy than conventional HPLC-UV methods, primarily due to reduced chemical interference (Gunes et al. 2024).

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Cellular permeability models in drug discovery and development allow the investigation of the permeability potential of drugs, the mechanism(s) of drug absorption, structure-transport relationships, and drug–food interactions (Han et al. 2006; Berginc et al. 2010; Volpe 2011; Awortwe et al. 2014). In the present study, the Caco-2 permeability model system was used to evaluate the modulatory effects of fat-soluble vitamins (VA and VE) on the intestinal permeability of EPR. Apparent permeability (P_{app}) values serve as quantitative indicators of transcellular transport efficiency, with higher values associated with greater absorption potential (Volpe et al. 2007). Conversely, decreased P_{app} values may reflect restricted transport due to physicochemical factors or transporter modulation, including P-gp (Hidalgo et al. 1989; International Transporter Consortium et al. 2010). Our study determined that the co-administration of VA and EPR resulted in an approximately 40% decrease in the P_{app} value. VE did not significantly alter EPR permeability. Furthermore, when both VA and VE were combined, EPR permeability showed a ~34% reduction. Collectively, these findings suggest that the primary modulatory effect causing the reduction in EPR permeability is predominantly attributable to VA. This effect may be attributed to acute, non-genomic interactions occurring at the intestinal epithelial level (Losel et al. 2003). Due to its lipophilic ester structure, VA can rapidly partition into the membrane lipid phase, thereby altering membrane organisation or sequestering the freely diffusible fraction of EPR at the apical interface. Such membrane-associated interactions can limit transcellular diffusion without necessitating the direct modulation of efflux transporter activity (Artursson and Karlsson 1991; Herr et al. 1999; Volpe et al. 2007; Escriba et al. 2008). Taken together, these findings suggest that the observed decrease in EPR permeability is more likely driven by membrane-level physicochemical interactions rather than direct modulation of P-gp-mediated efflux. Notably, P-gp inhibition would be expected to increase intracellular drug accumulation and thereby enhance apparent permeability, which is inconsistent with our findings (Lespine et al. 2009; Sharom 2011). In addition, significant modulation of P-gp expression is unlikely under the short experimental conditions used in this study, as transcriptional regulation typically requires longer exposure times (Tolson and Wang 2010). Given the highly lipophilic nature of both EPR and the tested vitamins,

it is more plausible that their interaction within the lipid bilayer alters the membrane properties and may promote drug sequestration, thereby reducing its transcellular diffusion (Wang and Quinn 2000; Atkinson et al. 2021; Radzin et al. 2023).

Vitamin A propionate (retinyl propionate) is a lipophilic retinyl ester form of VA, as described in comparative metabolic studies showing its distinct profile among retinoid esters such as retinyl palmitate (Bjerke et al. 2021). Retinyl esters are predominantly transported across Caco-2 monolayers via membrane partitioning and passive transcellular diffusion rather than carrier-mediated uptake, with a tendency for apical retention and limited basolateral efflux (Artursson and Karlsson 1991; Herr et al. 1999; During and Harrison 2007; Volpe et al. 2007). The lack of a comparable effect for VE in our study is consistent with existing literature indicating that P-gp inhibition is primarily associated with synthetic VE derivatives, such as TPGS, rather than natural α -tocopherol (Yu et al. 1999; Yang et al. 2018). While α -tocopherol and its analogues have been demonstrated to modify the membrane dipole potential (MDP) and subsequently influence the ligand-binding capacity of P-gp, our results under short-term exposure conditions support a mechanism driven by physicochemical and membrane-level interactions (Collnot et al. 2006; Davis et al. 2015). Therefore, the observed selectivity for VA may stem from its specific ability to perturb membrane packing or lipid ordering more effectively than natural α -tocopherol within the timeframe of our experiments.

As with other lipophilic compounds with limited oral bioavailability, the intestinal permeability of EPR can be effectively investigated using the Caco-2 model to identify membrane-level and intracellular factors restricting transepithelial transport, independent of classical efflux mechanisms (Wahlang et al. 2011; Ustun Alkan et al. 2014). Similar observations have been reported in studies proposing that fat-soluble vitamins may affect drug absorption and bioavailability through nutrient–drug interaction mechanisms (Renaud et al. 2024). From a veterinary pharmacotherapy perspective, such interactions could compromise therapeutic efficacy, particularly in animals receiving vitamin-enriched diets (Finno 2020).

EPR, a macrocyclic lactone, is known to be influenced by P-gp, and impaired P-gp function may increase systemic and central nervous system exposure, as demonstrated for ivermectin (Geyer et al. 2005;

Mealey et al. 2023), particularly in certain dogs with MDR1 mutations. However, EPR is generally considered to have a more favourable safety profile, with limited evidence of species-specific neurotoxicity in the literature (Lespine et al. 2009). In the present study, VA and VE decreased EPR permeability, suggesting that co-administration is unlikely to increase systemic exposure or toxicity risk. Accordingly, the likelihood of clinically relevant neurotoxic effects under these conditions appears low.

Our findings suggest that VA propionate, a commonly used VA ester in feed additives (EFSA 2013), may reduce the intestinal permeability of EPR in Caco-2 cell monolayers. Given the short incubation period (2 h), these effects are more plausibly attributed to acute alterations in membrane fluidity or tight-junction organisation rather than delayed genomic regulation of epithelial transport pathways. These findings emphasise that nutrient-drug interactions should be considered not only from a transporter-based perspective but also in terms of membrane-level physicochemical effects.

In this study, the effects of VA and VE on the intestinal permeability of EPR were evaluated using the Caco-2 cell monolayer model. Our findings demonstrated that both vitamins, particularly VA, reduced the apparent permeability of EPR. These results suggest that the observed reduction in permeability may be attributed to acute, membrane-level physicochemical interactions, such as alterations in membrane organisation or a reduced availability of the freely diffusible drug fraction at the intestinal interface. From a veterinary pharmacological perspective, these results indicate that lipophilic dietary supplements, particularly VA esters, may interfere with the intestinal absorption of orally administered EPR. Such nutrient-drug interactions warrant careful consideration in veterinary practice, as they may influence therapeutic efficacy and residue profiles in food-producing animals. Overall, this study provides experimental evidence supporting the relevance of membrane-mediated nutrient-drug interactions in veterinary pharmacotherapy and highlights the need to consider dietary composition when evaluating the oral use of antiparasitic agents.

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Conflict of interest

The authors declare no conflict of interest.

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