ABSTRACT

Antiparasitic drugs play a crucial role in protecting animal health and represent one of the most frequently used therapeutic groups in veterinary medicine. Due to the growing problem of parasite resistance to currently available active substances, there is an increasing need for novel compounds characterized by distinct mechanisms of action and favorable pharmacological properties. Isoxazoline derivatives constitute a new and rapidly developing class of antiparasitic drugs whose application has expanded in recent years from companion animals to selected livestock species, including laying hens. Although the European patent EP3878281B1 covers the potential use of fluralaner, sarolaner, lotilaner, and afoxolaner in poultry, pharmacokinetic data are currently available only for fluralaner. Consequently, the pharmacokinetics of the remaining isoxazoline derivatives in laying hens has remained unknown. Furthermore, it has not been elucidated whether efflux transporters, including P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BCRP/ABCG2), are involved in the pharmacokinetics of isoxazoline derivatives, despite their established role in the disposition of numerous xenobiotics, including many highly lipophilic drugs. Therefore, the present study was designed to address three research objectives: (1) to characterize the pharmacokinetics of selected isoxazoline derivatives, namely fluralaner, sarolaner, lotilaner, and afoxolaner, in laying hens and to determine whether significant differences in pharmacokinetic properties exist among these compounds in this species; (2) to evaluate the involvement of P-gp and BCRP in the pharmacokinetics of fluralaner in laying hens using elacridar as an inhibitor and oxytetracycline as a substrate of these transporters by analyzing changes in the fluralaner concentration–time profile; and (3) to optimize and revalidate an in-house ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method enabling the simultaneous and accurate determination of selected isoxazoline derivatives in plasma. The study was conducted in ISA Brown laying hens administered fluralaner, sarolaner, lotilaner, and afoxolaner intravenously (IV) and orally (PO) under a unified experimental design, enabling direct comparison of their pharmacokinetic parameters. The influence of the efflux transporters P-gp and BCRP on the pharmacokinetics of fluralaner was assessed following administration of elacridar, a dual P-gp/BCRP inhibitor, and oxytetracycline, a substrate of these transporters. Plasma concentrations of the investigated compounds were determined using a UPLC-MS/MS method, and pharmacokinetic analysis was performed using a non-compartmental approach. Direct comparison of fluralaner, sarolaner, lotilaner, and afoxolaner revealed significant differences in their pharmacokinetic profiles following both IV and PO administration. Among

the investigated compounds, sarolaner exhibited the highest systemic exposure (AUC) and the longest time to the last quantifiable concentration (t_{last}) after PO administration. In contrast, following IV administration, lotilaner was characterized by the highest clearance (Cl) and the highest elimination rate constant (k_{el}). Administration of elacridar resulted in increased AUC and bioavailability (F) of fluralaner, whereas oxytetracycline produced changes of a similar but less pronounced magnitude. As part of the methodological component of the study, a multianalyte UPLC-MS/MS method enabling the simultaneous determination of fluralaner, sarolaner, lotilaner, and afoxolaner in laying hen plasma was optimized and revalidated in accordance with EMA and FDA bioanalytical method validation guidelines. This study provides the first direct comparison of the pharmacokinetics of fluralaner, sarolaner, lotilaner, and afoxolaner in laying hens under unified experimental conditions. The obtained results allowed the following conclusions: (1) despite their close structural relationship, fluralaner, sarolaner, lotilaner, and afoxolaner do not constitute a homogeneous pharmacokinetic group in laying hens but differ with respect to absorption, distribution, and elimination. Sarolaner exhibits the greatest systemic exposure and the longest mean residence time, whereas lotilaner is characterized by the most rapid elimination; (2) the obtained findings indicate that efflux transport mechanisms may contribute to the bioavailability and pharmacokinetic profile of fluralaner; and (3) the validated multianalyte UPLC-MS/MS method enables the simultaneous determination of fluralaner, sarolaner, lotilaner, and afoxolaner in laying hen plasma, thereby facilitating comparative pharmacokinetic studies of these compounds.