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IN VITRO METABOLISM STUDY OF SALT OF THE ACID LASALOCID BY RAT HEPATIC LIVER MICROSOMES

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Abstract: The discovery of new drugs is deeply connected to innovations in the chemistry, pharmacology, molecular and cell biology, which improves the understanding of biochemical and physiological pathways, and molecular targets, making possible the discovery of new assets [1]. The advances in combinatorial synthesis and the use of high-throughput screening, a decrease by the pharmaceutical industry to develop drugs of natural origin was observed. However, with the decrease in the number of new drugs launched in the market in the past two decades, the pharmaceutical industry has been interested to reconsider natural products with proven biological activity in new targets. The lasalocid (commercial salt of lasalocid acid) is a carboxylic ionophore produced by *Streptomyces lasaliensis*. Ionophores are also classified as polyether antibiotics and exhibit a broad spectrum of bioactivity, varying from antibacterial to antiviral and, more recently, its cytotoxicity in tumor cells was discovered [2]. Although widely used in veterinary products little is known about the phase 1 metabolism of this substance. As mentioned above, this ionophore showed a promising antitumor activity, which, along with the lack of data on its metabolism in the face of cytochrome P450 enzymes, stimulates further investigation. To determine the *in vitro* enzymatic kinetics parameters, an HPLC method was developed and validated to quantify lasalocid in rat liver microsomes. All samples were separated on a reversed-phase C18 column using methanol:water (90:10, v/v, with 0.1 % of formic acid) as mobile phase. The method exhibited a linear range of 0.1 – 52.0 µg/L, with the following calibration curve: y = 0.0015x + 0.0009, r = 0.999. The lower limit of quantitation was 0.1 µg/L with an RSD below 12.2 %. The precision and accuracy were assessed for both within-day and between-day determinations; neither relative standard deviations (RSD %) nor relative errors (RER) exceeded a value of 15 %. The enzymatic kinetics parameters revealed a Michaelis–Menten profile, with V_{max} = 8.07 ± 0.72 µM/(mg protein/mL)/min and K_m 5.16 ± 1.34 µM. Employing a mammalian model, metabolism yielded three unreported monohydroxylated products (*m/z* 629). For the first time, the *in vitro* metabolism employing microsomes was demonstrated to be a suitable tool for enzymatic kinetics and for the determination of the produced metabolites of the lasalocid by a mammalian model.

References:

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