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THYMOL ANALYSIS IN CATTLE PLASMA SAMPLES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract: The use of medicinal plants as an alternative to drug treatment stimulates new research, and aroused interest in the application of the essential oil of *Thymus vulgaris* L. (Thyme) in the treatment of cattle as a possibility to decrease somatic cell count associated with intramammary infections, which reduce milk quality and cause damage to the dairy industry [1,2]. Thymol (2-isopropyl-5-methylphenol) is a volatile phenolic monoterpene being the majority compound of thyme and responsible for the main and most obvious biological activity reported in the literature, as antimicrobial agent [3]. This study aims to extract and quantify thymol in plasma samples from cattle through validated techniques headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). For the extraction of thymol in plasma we used a polydimethylsiloxane-divinylbenzene (PMDS/DVB) fiber, and determined that the following optimized conditions: 1 mL of plasma diluted in 9 mL of water, adding 1.5 g of NaCl, pre-heating the samples at 90 °C for 5 min, a stirring speed of 540 rpm, exposure of the SPME fiber to the headspace of the sample for adsorption of analytes for 40 minutes under the same conditions of temperature and agitation [4]. Then the analytes are desorbed from the fiber in the gas chromatograph interface to a mass spectrometer (Shimadzu, model QP2010). The ideal chromatographic conditions developed were achieved through a capillary column EN-5 MS (30m x 0.25mm x 0.25um) (SGE Analytical Science), starting at a temperature of 60 °C, and conditioned to increase by 6 °C/ min up to 160 °C, and then increasing 20 °C/min to 250 °C and remained at this temperature for 4 minutes, 25.17 minutes for a total running time. Helium was the carrier gas used in a flow rate of 1.4 mL/min. Injection occurred in the splitless mode for 1.5 minutes with detector at 250 °C. The source of impact ionization was set to 250 °C, and the mass analyzer operated in the mode SIM (Selected Ion Monitoring) selecting ions grouped by time, the first 4 to 12.8 minutes monitoring the ions m/z 150 and 163 to thymol; and second 12.8 to 25.17 minutes for the ions m/z 163 and 178 for the internal standard propofol. The analytical method was linear in concentrations from 0.25 to 75.0 ng/mL. The method was able to detect and quantify thymol at concentrations expected to be found in plasma from cattle treated with the essential oil of thyme.

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