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Abstract: An assay method to quantify ondansetron (OND), granisetron (GRA) and tropisetron (TRO) in goat plasma has been successfully developed and validated. This method procedure for the analysis of OND, GRA and TRO was involved of extracting samples with hydrophilic interaction liquid chromatography (HILIC) solid phase extraction (SPE) and determination by liquid chromatography coupled to tandem mass spectroscopy. An SPE method for the simultaneous extraction of OND, GRA and TRO with high efficiency and selectivity was developed. Prior to HPLC-MS/MS analysis, most of the sources of interference present in the supernatant after protein precipitation of plasma proteins was efficiently removed from the samples by the HILIC SPE treatment. For the quantification of OND, GRA and TRO in the samples, tandem mass spectrometry operating in positive electrospray ionization mode with multiple reaction monitoring was used. The calibration curve was performed in the range of 0.2-20 ng/mL for the target OND, GRA and TRO in goat plasma samples.