A simple and rapid Liquid chromatography-tandem mass spectrometry method for betamethasone measurement in human plasma venous blood and umbilical cord blood Kupa LVK[3], Romano P[1,2], Almeida REP[1,2], Duarte NJC[1,2], Chalom MY[1]. [1]Central Laboratory Division, [2]Laboratório de Investigação Médica (LIM 03), [3]Laboratório de Investigação Médica (LIM 17), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil

Background
HPLC-MS/MS is considered the gold standard for therapeutic drug monitoring (TDM) in several clinical settings to guarantee efficacy while avoiding toxicity. Antenatal betamethasone therapy is recommended as a standard of care for women at risk of preterm birth to reduce perinatal morbidity and mortality. We developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify betamethasone in human plasma from venous blood and umbilical cord blood.

Methods
Positive Electrospray Ionization was used in a triple quadrupole LC-MS/MS system at SRM Mode and yield consistent signals with selective transitions of m/z 393.2 -->373.1 for betamethasone and 398.2 --> 368.1 for the internal standard (betamethasone-d5). To each 150 µL of sample, 10 µL (500ng/mL) of internal standard, 200 µL of methanolic 2mM Zinc Sulfate solution and 200µL of pure methanol was added. Samples were centrifuged at 8000 rpm for 10 min and 15 µL of the extract supernatant were injected to a Hypersil Accucore C18 column (100 mm x 2.1 mm ID, 1.9 microns), eluted with a linear gradient of methanol-water containing each 2 mmol x L(-1) ammonium formate and 0,1% Formic Acid at a flow rate of 0.35 mL x min(-1). Validation studies were performed based on international standards.

Results
Betamethasone and its internal standard eluted consistently at 3.0 min. The calibration curve of peak area ratio (BET/ISTD) *versus* betamethasone concentrations was linear from 0.290 up to 218.3 ng/mL in venous blood ($r^2 = 0.998$) and from 1.18 up to 100.1 ng/mL in umbilical cord ($r^2 = 0.9981$). The method had a detection limit of 0.020 ng/mL and a lower limit of quantification of 0.29 ng/mL for venous blood and umbilical cord. Precision assay yield a coefficient of variation of 0.8 to 1.5% for intra-assay and 1.5 to 4.95% for inter-assay for both matrix samples at low, medium, and high concentrations. The accuracy was 100 to 121%, and the recovery was 91.2 to 114.3 % for low, medium, and high concentrations of betamethasone in both matrices ($r^2 = 0.9871$).

Conclusions
The method was successfully developed, validated and will be applied for TDM and pharmacokinetic studies.

Key words: Betamethasone; LC-MS/MS; plasma levels; Therapeutic drug monitoring