DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD UPLC-MS-MS FOR THE DETECTION OF ANTIRETROVIRALS IN AMNIOTIC LIQUID

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The first-line antiretroviral treatment to prevent HIV vertical transmission is the high activity antiretroviral therapy HAART composing of Lamivudine, LMV, Zidovudine, ZDV, Lopinavir, LPV and Ritonavir, RTV. The antiretrovirals concentrations and their effects in amniotic fluid during pregnancy have been little studied, only some effects are known to their exposure such as itochondrial dysfunction and hyperlactemia in neonate. The relationship between fetus exposure to antiretroviral drugs during pregnancy is not clear. The concentrations of the HAART in amniotic fluid it will allow us to understand the correlation between the adverse effects on the fetus during pregnancy, for which it is necessary to develop analytical tools such as liquid chromatography coupled to mass spectrometry in tandem.

METHODS

The detection was made by spectrometry in tandem by positive electrospray, the fragmentation patterns of drugs are as follows: LMV, 230.12-312.08, ZDV, 268.20-127.10, LPV, 629.50-447.35 and RTV, 721.50-296.20, the chromatography separation was carried out with an Acquity BEH C18 column 2.1 umx50mm, 1.7um, the mobile phase is made up of acetonitrile and formic acid 0.1%.

The injection was 3 uL and the running time was 2.80 minutes, with a flow rate of 0.3 mL-min. Proteins was precipitated by the addition of 300 uL of 0.1% formic acid in cold-iceacetonitrile to 100 uL of sample followed by centrifugation at 13000rpm for 10 min at 4C. The method was validated in accordance with the recommendations of both the United States Food and Drug Administration and the Mexican standards, NOM-177-SSA1-2013.

RESULTS

The concentration ranges wasng per mL: LMV, 125-3750, ZDV, 200-6000, LPV, 1.25-37.5 and RTV, 1.25-37.5. Inter-batch and intra-batch precision and accuracy was less than 15 per cent to according to national and international standars.

CONCLUSIONS

The methodology developed allowed simultaneous quantification of LMV, ZDV, LPV and RTV in amniotic fluid from positive-HIV pregnant women with precision and accuracy. The applied of this tool in the antiretroviral quantification will allow to establish the relations of the adverse effects on the fetus.