

Determination of Metformin Levels in Human Blood via LC/MS/MS

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Metformin is an anti-diabetic drug which belongs to biguanide class. It is the most prescribed oral anti-hyperglycemic agent used in managing the type II diabetes mellitus. It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues. Since metformin is a high polar compound, the extraction of metformin from human blood is somewhat complicated. Thus, a quick and high-through liquid chromatography mass spectrometry (LC/MS/MS) method has been developed and validated for the estimation of metformin in human blood with a simple sample extraction technique. Solid-phase extraction was used for sample preparation. Hereafter, it was followed by liquid chromatography tandem mass spectrometric analysis and an electrospray-ionization interface. The compound was analyzed using a shim-pack XR-ODS III (1.6 μ m, 2.0 mm I.D. x 50 mm) column with the mobile phase consisting of 10 mmol/L ammonium formate in 0.1% formic acid in methanol as solvent A and acetonitrile as solvent B with gradient elution mode at a flow rate of 0.3 mL/min for 10 min. A retention time of 0.387 min was observed for metformin. The method was validated as per the guidelines of Scientific Working Group for Forensic Toxicology (SWGTOX) for linearity, precision, accuracy, limit of quantification, limit of detection and recovery. All the results obtained were found to be within the acceptance limit. Hence, the developed LC/MS/MS method was successfully applied for the determination of metformin in human blood. Further, it was successfully employed to measure qualitatively metformin level in a sample obtained from a deceased person who had committed suicide by overconsumption of metformin.

Keywords: human blood, LC/MS/MS, metformin, method validation, solid phase extraction