

**P540**

### **Validation of HPLC method for analysis of adenine in plasma**

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**Introduction:** Adenine, a purine nucleobase, is an integral component of nucleic acids. It is also a constituent of the cellular energy provider adenosine triphosphate (ATP). Beside the cellular functions, adenine is commonly used to induce renal injury to produce acute and chronic renal failure models in rats and mice. It induces renal failure in most of the studies but there remain a need to establish a concentration-tissue damage relationship. Therefore, a HPLC method is validated for the measurement of adenine in plasma.

**Methods:** 1.0 mL adenine solution (0.1mg/mL) was mixed with 1.0 mL plasma and vortexed for 5 minutes followed by 10 minutes shaking. 15.0 mL acetone was added after settlement for 1.0 h. The mixture was then centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and evaporated to dryness. The residue was reconstituted with 5.0 mL phosphate buffer (100 nM, pH 8.0). C<sub>18</sub> solid phase cartridge (Waters, USA) was used for extraction. Cartridge was pre-conditioned with 2.0 mL of MeOH followed by 5.0 mL Millipore water. Spiked sample was passed through the cartridge with 0.5 mL flow rate followed by cartridge washing with 2.0 mL water at same flow rate. The cartridge was then dried by passing hot air and the drug eluted by 5.0 mL methanol containing 0.1% acetic acid at 0.5 mL of flow rate. The eluted methanol was concentrated under vacuum up to 0.5 mL and filtered through 0.22 µm membrane and used for HPLC analysis. 5.0 µL sample of adenine (0.1 mg/mL) was injected into the chromatographic system (2695 pump, Waters, USA). The separation was performed on Sunniest RP AQUA C<sub>28</sub> (250 x 46 mm, 5.0 µm) of ChromaNik, Japan at 27±1 °C. The mobile phase consisting water and acetonitrile (90:10, v/v) was delivered at a flow rate of 1.0 mL min<sup>-1</sup>. The detection of the analytes was carried out using UV detector (475 module, Waters, USA), at wavelength 225 nm.

**Results:** The obtained HPLC chromatograms of adenine in standard and plasma samples indicated good and fast separation. The values of retention factor was 0.50. The method was validated and found to be linear in the range of 0.01-1.0 mg/mL. The limit of quantification was 10.0 µg/mL. The precision (RSD) and the accuracy (percent error) values were 0.50-0.55 and 0.5-1.05, respectively. The extraction recoveries were more 80%.

**Conclusion:** The method was validated and found to be useful for analysis of adenine in plasma of human and any other animals.

**Keywords:** Adenine, Plasma, SPE, HPLC validation, C<sub>28</sub> Column.