

## Abstract

### **Bioanalytical method development for the investigation of role of pyridoxal isonicotinoyl hydrazone in isoniazid intoxication II.**

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HPLC/UV method was developed for analysis of isoniazid - INH, its main (acetylisoniazid - AcINH) and putative metabolites (pyridoxal isonicotinoyl hydrazone - PIH, pyridoxal isonikotinoyl hydrazone 5-phosphate – PIH5P), vitamins B<sub>6</sub> (pyridoxine - PN, pyridoxal - PL and pyridoxal 5-phosphate - PLP) using ZIC-*p*HILIC stationary phase. The following parameters were modified to assess their impact on the retention of these compounds: composition of mobile phase, percentage content of organic solvent, buffer concentration, pH, flow rate of mobile phase and column temperature. The best available separation of the compounds was achieved using 10mM ammonium formate (pH 3) and ACN (40:60, v/v), flow rate 0.10 ml/min and column temperature 25 °C.

Developed method was transferred to HPLC/ESI/MS. Experiments revealed that all analytes gave the higher signal in positive mode and the most abundant ions were the protonated molecules  $[M+H]^+$ . All analytes were determined in SIM (selected ion monitoring) mode at following *m/z*: INH 138, AcINH 180, PN 168, PL 170, PLP 248, PIH 287 and PIH5P 367.