



## Abstract

In this study a simple, rapid and selective RP-HPLC-DAD (Reverse Phase High Performance Liquid Chromatography with diode array detector) method is developed and validated for the simultaneous determination of illicit drugs from various energy drinks available in Pakistan. To increase the effectiveness of energy drinks numerous illicit drugs are added undeclared which cause lethal effects on health. The drugs under analysis were caffeine, niacin, folic acid and nicotinamide. The chromatographic separation was executed within the run time of 6 minutes using C18 column (250 mm x 4.6 mm, 5-  $\mu$ m particle) and gradient elution system consisting of methanol and 0.005M Ammonium Acetate buffer (pH 5.6). The gradient pattern was 40, 80, 40 and 40 with the flow rate 1mL/min at room temperature ( $25 \pm 2^\circ\text{C}$ ). The chromatogram was observed at 210 nm, 254 nm, 263 nm and 272 nm. Developed method was validated according to ICH guidelines. The linearity values of caffeine, niacin, folic acid and nicotinamide were 0.9988, 0.9983, 0.9977 and 0.9993 respectively. The LOD value of this developed method for caffeine was found to be 0.5 $\mu$ g/mL. The obtained values of LOD for niacin were 1.0  $\mu$ g/mL, folic acid 0.8 $\mu$ g/mL, nicotinamide 1.4 $\mu$ g/mL. The LOQ values were 3.5 $\mu$ g/mL for caffeine, 3.8 $\mu$ g/mL for nicotinamide, 2.1 $\mu$ g/mL for folic acid and 3.3 $\mu$ g/mL for niacin. After applying different stress conditions, different types of impurities along with other ingredients were separated at different wavelengths. 5 commercially available energy drinks were collected from local market and the developed method was applied on it. The findings affirmed the spiking of numerous undeclared illicit drugs in the energy drink samples.