The paper reports on the development of an accurate hydrophilic liquid interaction chromatography tandem mass spectrometry (HILIC-MS/MS) based stable isotope dilution analysis for the simultaneous quantitation of the food-derived bioactive pyridines trigonelline, nicotinic acid, nicotinamide, and N-methylpyridinium, as well as their key metabolites nicotinamide-N-oxide, N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide, N-methyl-4-pyridone-5-carboxamide, and N-methyl-2-pyridone-5-carboxylic acid in human plasma and urine. Precision of the stable isotope dilution analysis (SiDA) was 1.9(%) and 11.9(%) relative standard deviation (n = 6), and accuracy was between 92.4(%) and 113.0(%). The lower limit of quantitation (LLOQ) was 50 fmol (10 pmol/mL) injected onto the column for all analytes with the exception of N-methyl-2-pyridone-5-carboxylic acid and N-methyl-2-pyridone-5-carboxamide, for which an LLOQ of 100 fmol (20 pmol/mL) was found. The method was applied to monitor the plasma...
appearance and urinary excretion and to determine pharmacokinetic parameters of the bioactive pyridines as well as their metabolites in a clinical human intervention study with healthy volunteers (six women, seven men) after oral administration of 350 mL of a standard coffee beverage. Trigonelline plasma levels increased from 160 nmol/L to maximum concentrations of 5479 (males) or 6547 nmol/L (females), and N-methylpyridinium plasma levels raised from virtually complete absence to maximum values of 777 (females) or 804 nmol/L (males) within 2-3 and 1-2 h after coffee consumption, respectively. The high plasma levels of N-methylpyridinium found after coffee consumption clearly demonstrate for the first time that this cation is entering the vascular system, which is the prerequisite for biological in vivo effects claimed for that compound. In contrast, the coffee intervention did not significantly influence the plasma concentrations of N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-5-carboxamide, the major niacin metabolites. Within 8 h after coffee intervention, an urinary excretion of 57.4 +/- 6.9(%) of trigonelline and 69.1 +/- 6.2(%) of N-methylpyridinium was found for the male volunteers, whereas females excreted slightly less with 46.2 +/- 7.4(%) and 61.9 +/- 12.2(%) of these pyridines.