A straightforward stable isotope dilution analysis (SIDA) for the quantitative determination of trigonelline, nicotinic acid, and nicotinamide in foods such as coffee, as well as in biological samples by means of LC-MS/MS (MRM) has been developed. The coefficients of variation for their quantitative analysis in a coffee sample were 2.1{%} for trigonelline, 1.1{%} for nicotinic acid, and 3.1{%} for nicotinamide, and recovery experiments showed good results between 98.5 and 104.5{%}. Application of this SIDA for the quantification of trigonelline, nicotinic acid, and nicotinamide in coffee samples of different roasting degrees revealed a drastic degradation of trigonelline as well as the generation of nicotinic acid accounting for 4-6{%} of the initial trigonelline content, whereas nicotinamide remained rather constant at a low level. Besides the analysis of coffee samples, the feasibility of the developed SIDA was verified by analysis of other foods including breakfast cereals, rice, liver, and herring, as well as human urine and plasma samples.