## Abstract

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Caffeine is a xanthine alkaloid acting like a stimulant of heart and central nervous system. Quantification of caffeine in coffee drinks is significant to show how much of caffeine was in each cup which has been taken per day prior to prevent a caffeine overdose. The development of high-throughput sequential injection analysis (SIA) spectrophotometric assay for the determination of caffeine in coffee drinks was performed. Sample was treated with carrez reagent for matrix suppression followed by filtration thereafter analyte was isolated from organic acids by a short C18 monolithic column (10x4.6mm). The flow rate of the separation step was 10  $\mu$ L s<sup>-1</sup> with 10 % v/v of methanol as the mobile phase. Caffeine was detected at 274 nm. The influence of main parameters affecting the quantification of caffeine was optimized. Duration of one sample analysis was 15 minutes. During one hour it was possible to analyze 4 samples. Linear range was 1 - 15 mg L<sup>-1</sup> and determination coefficient ( $r^2$ ) was 0.9969. The limit of detection (LOD) and limit of quantification (LOQ) were 0.128 and 0.425 mg  $L^{-1}$ , respectively. The relative standard deviation (RSD) was 3.58 % ( $n = 12, 10 \text{ mg L}^{-1}$ ). Under optimal conditions, the method was successfully applied to determine caffeine in different real samples including the soluble coffee, coffee from espresso machine and brewed-coffee drinks.