

Quantification of methylxanthines (theobromine, theophylline and caffeine) in chocolate, tea and coffee-based beverages by HPLC

Abstract

This study was conducted to determine the concentration of methylxanthines in chocolates, tea and coffee-based beverage and to compare the concentration in the sample with the safe limit. A preliminary study was conducted via questionnaires on 50 respondents from the group of children and adults around Kota Kinabalu. Based on the preliminary study, it found that the brand for chocolate, tea and coffee-based beverage that were most frequently taken is Milo (78%), Lipton (37%) and Nescafe (58%) in the form of products consisting of natural non-mix powder (50%) and 3 in 1 pre-mixed powder (50%). Laboratory studies for the quantification of methylxanthines (theobromine, theophylline and caffeine) in chocolate, tea and coffee-based beverages are conducted using High Performance Liquid Chromatography (HPLC). Methylxanthines compounds was determined by using Synergy Hydro-RP C18 column (150x4.6mm) with a column temperature of 40°C, the mobile phase consisting of methanol:water (30:70), flow rate 1.0 mL/min and injection volume of 20µL. Linear regression equation for standard dilution series of theobromine in the calibration curve graph is $y=1.68x+0.0093$ with r^2 value=0.9553, the equation for theophylline is $y=1.7072x+0.0098$ with $r^2=0.93$ and the equation for caffeine is $y=1.6493x+0.0092$ with $r^2=0.9315$. The average concentration of theobromine in original chocolate samples is 33.6µg/mL, chocolate mix 39.2g/mL, original tea 43.5g/mL, tea mixture 1.8g/mL, original coffee 71.9g/mL and coffee mixture 8.3g/mL. The average concentration for theophylline in the original tea is 63.7g/mL, original coffee 34.8g/mL and coffee mixture 0.2 g/mL. No theophylline compounds detected in the samples of original chocolate, chocolate mixture and tea mixture. Meanwhile, the concentration of caffeine in the original chocolate is 2.5g/mL, chocolate mixture 2.8g/mL, natural tea 246.2g/mL, tea mixture 30.6g/mL, original coffee 198.7g/mL and coffee mixture 38.4g/mL. This study shows that the concentration of methylxanthines in chocolate, tea and coffee-based are low, not exceeding safe limits permitted.