

Optimized Simultaneous Determination of Theobromine and Caffeine in Fermented and Unfermented Cacao Beans and in Cocoa Products Using Step Gradient Solvent System in Reverse Phase HPLC

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Abstract : Fast, reliable and simultaneous HPLC analysis of theobromine and caffeine in cacao and cocoa products was optimized in this study. The samples tested were raw, fermented, and roasted cacao beans as well as commercially available cocoa products. The HPLC analysis was carried out using step gradient solvent system with acetonitrile and water buffered with H₃PO₄ as the mobile phase. The HPLC system was optimized using 273 nm wavelength at 35 °C for the column temperature with a flow rate of 1.0 mL/min. Using this method, the theobromine percent recovery mean, Limit of Detection (LOD) and Limit of Quantification (LOQ) is 118.68(±3.38)%, 0.727 and 1.05 respectively. The percent recovery mean, LOD and LOQ for caffeine is 105.53(±3.25)%, 2.42 and 3.50 respectively. The inter-day and intra-day precision for theobromine is 4.31% and 4.48% respectively, while 7.02% and 7.03% was for caffeine respectively. Compared to the standard method in AOAC using methanol in isocratic solvent system, the results of the study produced lesser chromatogram noise with emphasis on theobromine and caffeine. The method is readily usable for cacao and cocoa substances analyses using HPLC with step gradient capability.

Keywords : cacao, caffeine, HPLC, step gradient solvent system, theobromine

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