

## Documents

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**High performance liquid chromatographic method optimized by Box-Behnken design model to determine caffeine in pharmaceutical preparations and urine samples**

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### Abstract

A UV-HPLC method optimized by Box-Behnken design model was developed to determine caffeine in pharmaceutical preparations and urine samples. The chromatographic conditions followed were mobile phase: methanol/water/ citrate buffer (pH 4.6) (40:25:35, v/v/v), Acclaim™ Dionex C18 column (ODS 100A°, 5 µm; 4.6 × 250 mm), flow rate (0.9 mL min<sup>-1</sup>), column temperature (30 °C) and UV-detection wavelength (204 nm). The chromatographic variables: pH (A), % methanol fraction (B), flow rate (C) and column temperature (D) were optimized at 50 µg mL<sup>-1</sup> caffeine using BBD model. The chromatogram resulted in the asymmetry factor (1.23), theoretical plate 13,786 and retention time (5.79 min). The proposed HPLC method's greenness point was assessed by Analytical Eco-scale and found to be 78 (as per guidelines, ranked as excellent). The linearity was ranged from 2.0 to 70 µg mL<sup>-1</sup> with coefficient of correlation ( $r = 0.999$ ) and detection limit of 0.19 µg mL<sup>-1</sup>. The proposed method was developed successfully and applied for the assay of active caffeine in pharmaceutical preparations and urine samples. The % recovery obtained by both (proposed and reference) methods ranged from 99.98 to 100.05 % followed the compliance ( $100 \pm 2$  %) with Canadian Health Protection regulatory guidelines. The performance of the proposed method was compared with published papers and found to be acceptable and superior. The proposed method was quite effective as the reference method, and hence can be used as an alternative method for the assay of active caffeine in pharmaceutical preparations and urine samples. © 2024 Elsevier B.V.

### Author Keywords

Analytical Eco-scale Greenness; Box-Behnken Design; Caffeine; UV-HPLC

### Index Keywords

Chromatographic analysis, High performance liquid chromatography, Methanol, Regulatory compliance; Analytical eco-scale greenness, Box-Behnken design, Column temperature, Design models, High-performance liquid chromatographic methods, HPLC method, Pharmaceutical preparations, Reference method, Urine sample, UV-HPLC; Caffeine; buffer, caffeine, citric acid, methanol, water, caffeine, drug, methanol; analysis of variance, Article, assay, Box Behnken design, chromatography by mobile phase, comparative study, controlled study, error, flow rate, high performance liquid chromatography, limit of detection, limit of quantitation, molecular stability, pH, response surface method, retention time, solution and solubility, temperature, ultraviolet radiation, ultraviolet visible spectroscopy, uncertainty, urine sampling, validation study, Canada, high performance liquid chromatography, procedures; Caffeine, Canada, Chromatography, High Pressure Liquid, Methanol, Pharmaceutical Preparations

### Chemicals/CAS

caffeine, 58-08-2; citric acid, 126-44-3, 5949-29-1, 77-92-9, 8002-14-0; methanol, 67-56-1; water, 7732-18-5; Caffeine; Methanol; Pharmaceutical Preparations

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